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EXPERIMENTAL STUDIES ON THE DURATION OF LIFE. X. THE DURATION OF LIFE OF DROSOPHILA MELANOGASTER IN THE COMPLETE ABSENCE OF FOOD[1]

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IN the "Biology of Death" Pearl (52) advanced the idea that the inherent potentiality of the organism in respect of longevity was determined by its individual, inborn physico-chemical *organization,* using this word in the sense which has been so thoroughly developed by Henderson (65) in his "Order of Nature." It was further suggested that the actual realization or expression of the inborn potentiality of longevity was in major degree a function of the environment in which the life was lived. The environment determines in considerable part the rate at which the vital resources are used up. Since this suggestion was first made a good deal of evidence not then in hand has accumulated which indicates that the conception is a sound one (*cf.* particularly VII (57) and VIII (62) of this series).

Normally the living organ gains its energy for the conduct of life, and material for the repair, within limits, of the wastage of its tissues in the business of living, by the process of taking food. In other words the regular re-winding of the vital clock is accomplished by feeding. If this renewal or re-winding process is defective in any particular the result will be to shorten life below what

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would have been attained under more perfect metabolic functioning. But always when we study duration of life under normal conditions we are dealing with the combined effects of two variable complexes, inborn organization, on the one hand, and environment, including renewal of available energy and substance by food, on the other hand. Suppose now we eliminate the effect of as much as possible of this second complex experimentally. We shall then be in a better position to estimate how much of the normal variation in duration of life observed among different individuals is due to difference in their inborn make-up, their physico-chemical organization.

How can this be done experimentally? Clearly by doing two things: (a) preventing completely access to food of any sort, (b) keeping temperature, moisture and as many other variables of the physical environment as possible, constant throughout life. Such differences amongst individuals in respect of duration of life as then appear will be almost wholly due to innate protoplasmic, structural, constitutional differences, since the small residue of uncontrolled environmental variables will be unimportant ones, trivial in their effect upon the organism. The form of the life curve so obtained will be in a sense that which is basic to the species or variety. The idea of controlling the food factor of the environmental complex by starvation is methodologically of importance. It is extremely difficult to ensure experimentally that two different animals get identical food, quantitatively and qualitatively. But, with some care, it is easy to see that neither gets any food at all. In short, by complete starvation we can make constant the most difficult of all environmental variables to control accurately in an experiment.

These considerations suggest that such starvation life curves may furnish a powerful analytical tool for the more penetrating study of the biology of life duration. Consider specifically the case of *Drosophila*. We have shown in this laboratory (57), (62), (66) that certain genetic constitutions are invariably associated with cer-

tain definite forms of life curves. Wild type *Drosophila* has a characteristic life curve with a definite absolute mean duration of life, and with a characteristic shape. These attributes of the life curve are constant under constant environmental conditions. Under the same environmental conditions flies which carry the gene for the recessive wing character *vestigial,* whether alone and pure, or in combination with other mutant genes, exhibit life curves which differ widely in every important respect from the curves which describe the duration of life of wild type flies. They have a much shorter absolute duration of life than the wild type and the shape of the life curve when put upon a comparable basis by measuring age in centiles of the equivalent life spans (*cf.* Pearl (61)) is widely different from that for wild type flies. All this is clear. But do these differences depend upon (a) differences in the inborn physico-chemical organization, solely and *per se,* of the two sorts of flies, or upon (b) differences in the effective reactions of these two kinds of flies to the *same* environment, including most particularly food? Or, to put the experimental question, will wild type and vestigial strains of *Drosophila* show the same kind of differences in their life curves when these curves are determined under conditions of complete starvation that they do when both sorts of flies are fed the same kind of food? It is the purpose of this paper to present experimental evidence answering this question.

If the answer to this last question should turn out to be affirmative, it is evident that starvation life curves would offer a neat and time-saving method of studying life duration. For if the starvation life curve is merely a sort of foreshortened replica of the normal life curve of fed individuals, it will be clearly possible to accumulate data rapidly for many different forms. One can think of general facts of natural history that suggest that there is a correlation of this sort. The mouse is the shortest lived mammal. Its ability to endure starvation is extremely limited. In some experiments made in this

laboratory a number of years ago the longest time we were able to keep a wild mouse alive without food (but with water) was about 18 hours. This accords reasonably with the experience of others on the point. On the other hand, snakes, especially the larger ones, are known to be normally long lived. Wall (67, p. 63) states that the python in captivity will sometimes refuse food for long periods, "and without suffering perceptibly. Ferguson records one that fasted for over a year in the Trivandrum gardens, but changed its skin more than once, and always looked glossy and in perfect health." Many other observations of similar import might be cited.

There is little in the literature regarding duration of life in insects under conditions of starvation that has any particular pertinence to our present inquiry. Important data are those of the Raus (64, 68, 69) on the duration of life in saturniid moths which do not take food in the imaginal stage. In the preceding paper in this series we have prepared the l_x line, on a centile age base, calculated from their data for *Telea polyphemus*. It shows a form of curve intermediate between the wild *Drosophila*-man-*Proales* type of life curve and that characteristic of vestigial *Drosophila*. But of the greatest significance is the fact that, in the absence of food, there is in *Telea* an essentially similar amount and kind of individual variation in duration of life to what there is in normally fed individuals of other forms of life. This is shown by the similarity in form of the l_x curve for *Telea polyphemus* and for wild *Drosophila* or man, for example.

Vinokuroff (70) gives the mean duration of life of *Musca domestica* when starved without water as 1.3 days, and when starved, but given water, as 1.8 days.

Lutz (13) gives rather extensive data on the duration of life of *Drosophila* given water but not food. His data will be compared in a later section with those obtained in the present study.

Barrows (71, p. 517) incidentally mentions that *Drosophila* supplied with distilled water, but given no food will

not survive much longer than 24 hours. "If they are kept without food much longer than this, they begin to die and few survive sixty hours."

Holmes (72) studied the effect of starvation of the larvae of *Drosophila* upon the sex ratio, but gives no data on the influence of starvation upon the duration of imaginal life.

Kopeć (73) has lately published the results of extensive studies on the effect of intermittent starvation of the caterpillars of *Lymantria dispar*. The moths do not take food in the imaginal stage. "Starvation of caterpillars has no distinct effect on the duration of the imaginal stage." But *total* duration of life, from hatching to death, is prolonged by the intermittent starvation because of the extension of larval life. This prolongation may amount to 25 to 30 per cent. relative to the controls. Interesting and valuable as these observations are, they are concerned with an essentially different problem than that of the present study.

Glaser (75, 76) has recently contributed some extremely interesting and valuable experimental results regarding the effect of different food conditions upon duration of life in several species of flies. He notes that in the total absence of food *Musca domestica* lives only from one to two days. The numbers dealt with in these particular starvation experiments were small (12 flies all told) and it is impossible to construct a life table on such a meager basis. In general Glaser's experiments have been directed towards quite different problems than that of starvation.

Methods

To carry out critically an experiment on duration of life under starvation involves a number of difficulties. For the particular problem here dealt with it was necessary to have a large number of flies all emerging from pupal to imaginal life at about the same time, and under such conditions that they could not possibly get any food at all as imagoes. To attain this end the flies for these

experiments were reared in glass tubes 3 cm. in diameter and 11.5 cm. long. Through the larval and pupal periods the lower end of the tube was closed with a cork stopper on which a layer of standard food (27) of the usual depth had been poured. The top was closed by a cotton plug as usual. At the end of eight days, when the parent flies were removed from the tubes, the bottom cork and attached food were also removed and replaced by a clean cork. During this eight-day period most of the larvae had crawled out of the food and pupated on the sides of the tube, just as in the ordinary breeding bottle. Of course in this way some of the progeny were lost—those which had not pupated, or which had pupated on or near the surface of the food—but since all that was desired was to obtain a large sample of flies, raised under uniform conditions, all emerging within a short interval of time and having no food in the adult state, this was no objection to the method. Pupae could, of course, have been individually removed from ordinary breeding bottles to clean dry bottles for hatching, but this method would have taken much more time, besides introducing the possibility (indeed certainty in many cases) of injuring the pupae in handling. Progeny flies were removed every six hours, etherized, and counted out into clean, empty one ounce vials, in the different density groups. Each interval's hatch was distributed proportionately among the various densities.

The next point to be considered was that of moisture. It was obviously undesirable to run the duration of life tests in an absolutely dry atmosphere, because in addition to the starvation there would then be superimposed a desiccation effect due to evaporation of water from the flies' bodies. On the other hand, it was equally undesirable to have standing water where the flies could get at it. To that there are various objections. One important one is that it is practically impossible to furnish water to flies and not have the water contain some nutritive material in solution or suspension, particularly after

the first fly has been in contact with it. It should be realized when one is working with very small animals that any experiment which states that drinking water is furnished to otherwise starved individuals is really not a starvation experiment at all, but an experiment in feeding dilute food, unless the most extraordinary and practically impossible chemical precautions are taken. The theoretically most desirable procedure would seem to be to keep the flies during the experiment in an atmosphere so humid that there would be no significant evaporation from their bodies, and at the same time not sufficiently saturated to precipitate water in the tubes so that the flies could drink it. This state of affairs would prevent any desiccation effect from complicating the results, and also keep the flies from getting minute but real amounts of food in the guise of drinking water. After some preliminary experimentation the conditions desired were realized in the following manner.

All the duration of life bottles were kept in one electric incubator maintained at 25° C. The atmosphere was kept moist by trays of wet sand placed in the bottom of the incubator. Dry and wet bulb thermometers kept in the incubator throughout indicated that the relative humidity was held practically constant at about 80 per cent. This prevented any significant desiccation of the flies, as indicated by the condition of the dead ones. At the same time no standing drops of water condensed in the tubes.

The dead flies were removed and recorded every six hours, preliminary experiments having shown the total life span to be so short that several observations daily were needed to give enough points to construct the life tables.

Six hundred and fifty tubes were all started the same day, May 3, 1923, with three pairs of flies in each—300 tubes of Old Falmouth stock, line 107, and 350 tubes of pure vestigial stock. The parents and food were removed May 12, 1923. The flies had begun emerging May 14; the tubes were all emptied at 8 A. M., May 14, and this taken

as a starting point, that is, these first flies whose age was not accurately known were discarded, but the flies were removed at 2 P. M. and at 8 P. M., May 14, and at 2 and 8 A. M. and 2 P. M., May 15. By that time over 500 flies of exactly known age had been obtained for each density group of each stock and these were taken to be large enough samples for the experiment.

For help in carrying through the six hourly observations over more than four days continuously we are indebted to Dr. Mary Gover and Mr. James Krucky.

The plan of the experiments was as follows: approximately equal numbers of each sex of two stocks as above described were run in three density series; one of five flies per one ounce vial, one of 50 flies per one ounce vial, and one of 100 per one ounce vial. Other experiments (Pearl and Parker, 49, 74) had indicated that these densities represented conditions (a) well below, (b) approximately at and (c) well above the optimal density of population for wild type *Drosophila* at 25° C.

Data

The survivorship distributions, on the basis of 1,000 flies starting together at emergence, are given in Tables I and II.

TABLE I

SURVIVORSHIP DISTRIBUTIONS OF DROSOPHILA IN ENTIRE ABSENCE OF FOOD

Age (in hours)	Wild Type (Line 107)						Vestigial					
	Density 5		Density 50		Density 100		Density 5		Density 50		Density 100	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
3	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
9	996	996	997	998	997	997	1,000	997	990	987	1,000	1,000
15	989	989	986	980	988	981	997	993	977	967	997	997
21	986	978	979	973	981	978	986	969	970	961	997	991
27	967	968	976	936	978	966	972	938	961	935	977	966
33	917	918	927	922	960	944	923	903	924	908	944	948
39	790	806	729	837	810	897	784	831	786	866	812	914
45	511	570	319	631	498	803	422	731	536	788	545	840
51	192	208	101	329	153	516	192	483	178	618	155	637
57	62	61	21	149	12	297	42	214	23	294	23	323
63	7	11	0	58	3	88	10	45	0	59	6	111
69	0	0	—	14	3	13	0	14	—	3	0	18
75	—	—	—	3	0	3	—	3	—	0	—	—
81	—	—	—	0	—	0	—	0	—	—	—	—
Absolute No. of flies	276	279	288	295	321	320	287	290	304	306	341	325

TABLE II

SURVIVORSHIP DISTRIBUTION OF DROSOPHILA IN ENTIRE ABSENCE OF FOOD. BOTH SEXES TOGETHER

Age (in hours)	Wild (Line 107)			Vestigial		
	Density 5	Density 50	Density 100	Density 5	Density 50	Density 100
3	1,000	1,000	1,000	1,000	1,000	1,000
9	996	995	997	998	989	1,000
15	989	983	984	995	972	997
21	982	976	980	977	966	994
27	968	955	972	955	948	971
33	917	925	952	913	916	946
39	798	784	853	808	826	862
45	541	477	651	577	662	689
51	200	216	334	338	398	390
57	61	86	154	128	159	170
63	9	29	45	28	30	57
69	0	7	6	7	2	9
75	–	2	2	2	0	0
81	–	0	0	0	–	–
Absolute No. of flies	555	583	641	577	610	666

Before taking up the discussion of these distributions it is desirable to have it have the chief biometric constants of the mortality. These are given in Tables III and IV.

TABLE III

BIOMETRIC CONSTANTS FOR DURATION OF LIFE OF STARVED FLIES. SEXES SEPARATE

Density (flies per bottle)	Wild type. Line 107					
	Mean (hours)		Standard deviation (hrs.)		Coefficient of Variation	
	♂	♀	♂	♀	♂	♀
5	44.50 ± .36	45.03 ± .36	8.77 ± .25	8.91 ± .25	19.70 ± .59	19.79 ± .59
50	42.21 ± .31	46.94 ± .44	7.69 ± .22	11.08 ± .31	18.23 ± .53	23.61 ± .69
100	44.30 ± .29	50.89 ± .40	7.64 ± .20	10.73 ± .29	17.24 ± .47	21.09 ± .59
	Vestigial					
5	43.97 ± .33	48.72 ± .45	8.24 ± .23	11.31 ± .32	18.74 ± .55	23.22 ± .68
50	44.07 ± .35	50.31 ± .47	8.95 ± .24	12.12 ± .33	20.31 ± .58	24.09 ± .69
100	44.74 ± .27	52.47 ± .37	7.30 ± .19	9.98 ± .26	16.31 ± .43	19.03 ± .52

TABLE IV

BIOMETRIC CONSTANTS FOR DURATION OF LIFE OF STARVED FLIES. SEXES COMBINED

	Mean (hours)		Standard deviation (hrs.)		Coefficient of Variation	
	Wild type	Vestigial	Wild type	Vestigial	Wild type	Vestigial
5	44.77 ± .25	46.36 ± .29	8.84 ± .18	10.19 ± .20	19.76 ± .42	21.97 ± .46
50	44.60 ± .28	47.20 ± .30	9.85 ± .19	11.11 ± .21	22.08 ± .46	23.53 ± .48
100	47.59 ± .26	48.51 ± .25	9.88 ± .19	9.53 ± .18	20.75 ± .41	19.64 ± .38

Mean Duration of Life under Complete Starvation

The first outstanding result of the experiment is that *under complete starvation the duration of life is substantially the same or even longer in vestigial than it is in wild type flies.*

Considering the means of Table III we have the following system of differences:

Males. *Vestigial mean—wild type mean.*

Density 5; 43.97 — 44.50 = — .53 ± .49 (1.1)
Density 50; 44.07 — 42.21 = + 1.86 ± .47 (4.0)
Density 100; 44.74 — 44.30 = + .44 ± .40 (1.1)

Females. *Vestigial mean—wild type mean.*

Density 5; 48.72 — 45.03 = + 3.69 ± .58 (6.4)
Density 50; 50.31 — 46.94 = + 3.37 ± .64 (5.3)
Density 100; 52.47 — 50.89 = + 1.58 ± .55 (2.9)

Of the six differences five are positive in sign, signifying that the vestigials had a longer mean duration of life than the wild type. Three of the differences are probably not significant, while the other three probably are, as they are four or more times as large as their probable errors (the figures in parenthesis give the values of the ratio Difference/Probable Error of Difference).

Taking the mean of Table IV, where the sexes are combined in the computations we have the following differences:

Vestigials mean—wild type mean.

Density 5; 46.36 — 44.77 = + 1.59 ± .38 (4.2)
Density 50; 47.20 — 44.60 = + 2.60 ± .41 (6.3)
Density 100; 48.51 — 47.59 = + .92 ± .36 (2.6)

Here the differences are all positive. We believe that the whole system of differences, having regard for the probable errors involved, is such as to warrant the conclusion that the mean duration of life under complete starvation is certainly *not shorter* in vestigial flies than in wild type flies. It is *probably* slightly longer, but we have no desire to stress this difference in the positive direction. It may be taken merely to strengthen the conclusion that the vestigials do not have a shorter life under starvation than the wild type flies.

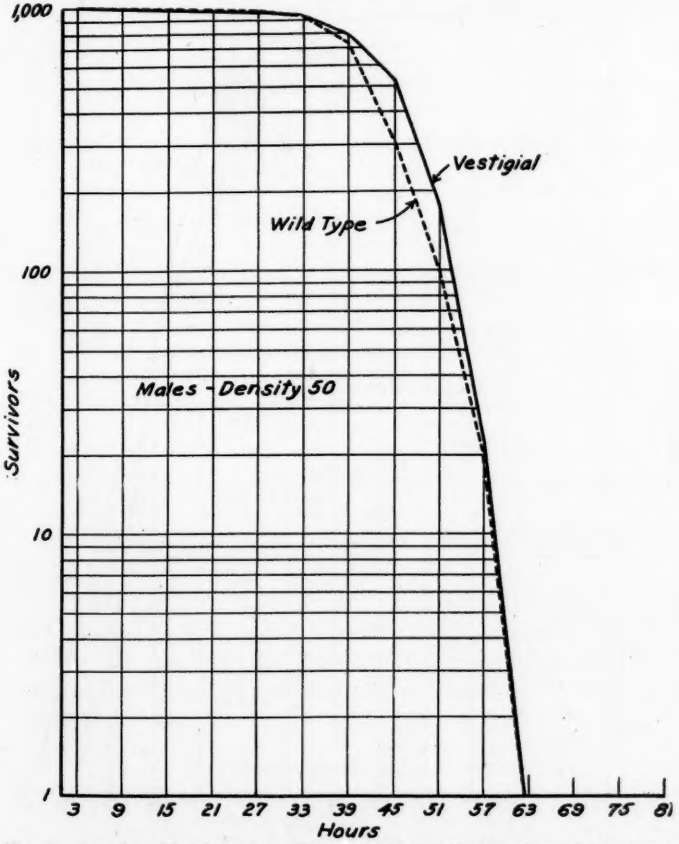

FIG. 1. Survivorship lines for wild type (broken line) and vestigial (solid line) *Drosophila* males, under complete starvation.

This result is also shown in a striking manner if we compare the survivorship lines of Tables I and II. This is done graphically in Figs. 1 to 3, inclusive. It is not necessary to show the lines for all three densities. The comparisons for one density, namely, 50, will sufficiently demonstrate this point.

It is evident that under the conditions of complete starvation that obtained in these experiments the vestigial flies lived quite as long, or a little longer, than the normal, wild type flies. This result is in marked contrast

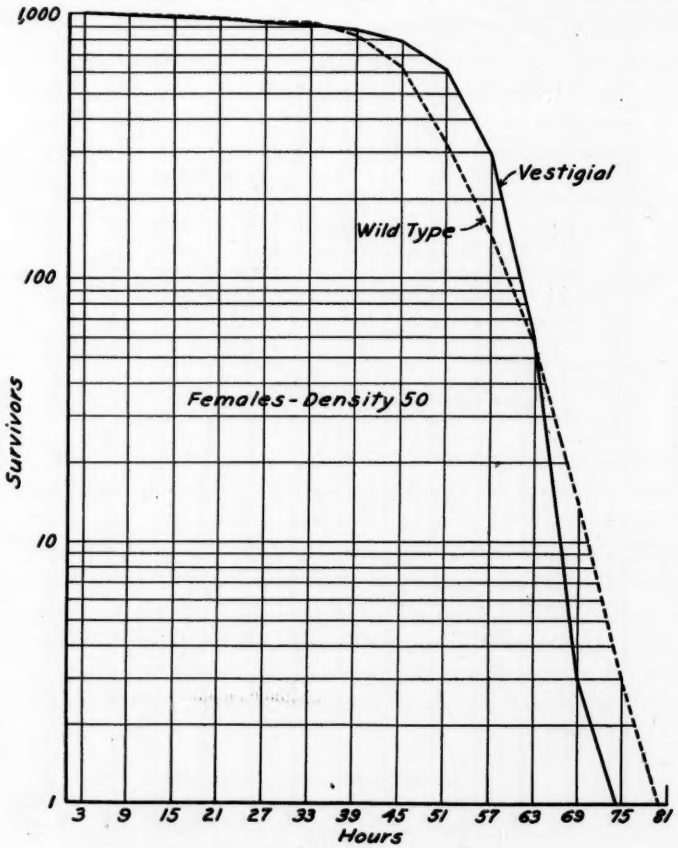

FIG. 2. Like Fig. 1, but for *females*.

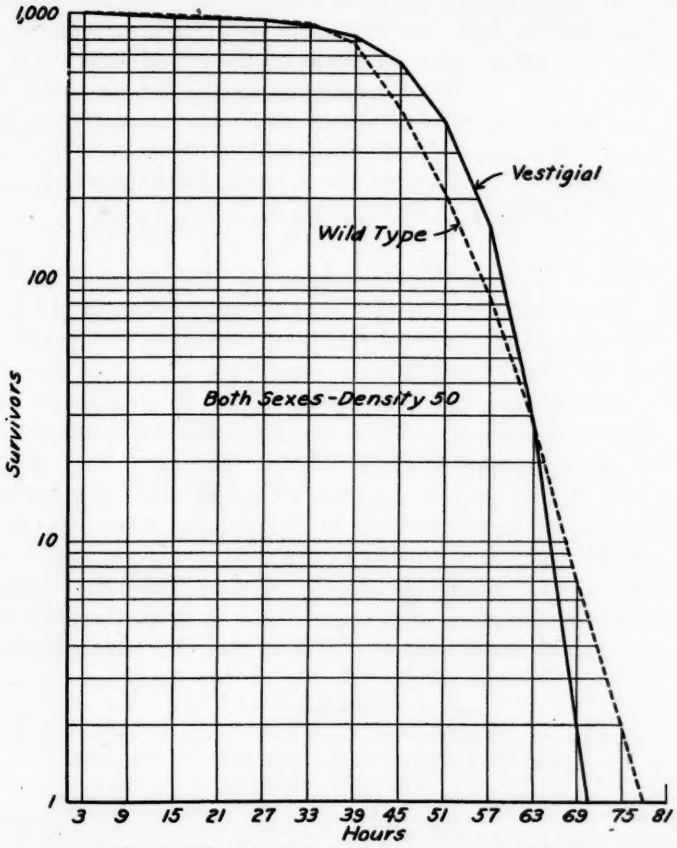

FIG. 3. Like Fig. 1, but for both sexes combined.

with what happens under normal conditions of feeding, as we have shown in a number of papers, and particularly with all the refinement of a life table in the study preceding this (66), the normal expectation of life at emergence is:

For wild type ♂♂, 45.8 days
For wild type ♀♀, 48.0 days
For vestigial ♂♂, 14.1 days
For vestigial ♀♀, 19.8 days

In other words, the mean duration of imaginal life, or expectation of life at emergence, is approximately *three times* as great, under normal feeding conditions, in the wild type fly as it is in pure vestigials. Under complete starvation the two are nearly identical in absolute duration of life, and what slight advantage there is favors the vestigials. What we believe to be the significance of this result will be discussed farther on in the paper.

Variation in Duration of Life under Complete Starvation

An examination of the last columns of Tables III and IV shows that the coefficients of variation for duration of life under conditions of complete starvation are all *under* 25 per cent., and a half of the 18 coefficients are under 20 per cent. These values indicate a *much lower relative variability in duration of life under starvation than under normal feeding.* This is demonstrated by comparing the coefficients of variation in Tables III and IV with any that we have published in earlier papers in this series. Take for example the coefficients of variation for duration of life in inbred lines as given in the second of these studies (32). There are given in that

TABLE V

COEFFICIENTS OF VARIATION IN DURATION OF LIFE IN (*a*) LINE BRED FLIES UNDER NORMAL FEEDING CONDITIONS, AND (*b*) FLIES UNDER COMPLETE STARVATION

Magnitude of coefficients of variation (in per cent.)	*a.* Under normal feeding	*b.* Under starvation
10–19	–	9
20–29	1	9
30–39	8	–
40–49	5	–
50–59	2	–
60–69	3	–
70–79	1	–
80–89	1	–
Totals	21	18

paper 21 coefficients of variation in duration of life. These are distributed in magnitude as shown in Table V. The distribution of the coefficients of Tables III and IV of this paper is inserted for comparison.

These two distributions are shown graphically in Fig. 4.

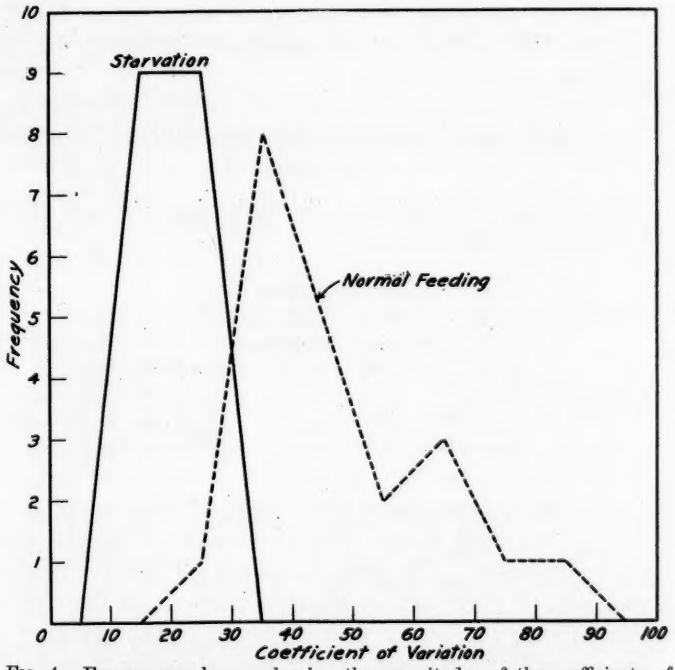

FIG. 4. Frequency polygons showing the magnitudes of the coefficients of variation in duration of life of *Drosophila* under starvation (solid line), and normal feeding (broken line).

The much reduced relative variability in duration of life under starvation is apparent. In fact it may be said that *roughly* one half of the variation observed among individual flies in the duration of life disappears when the varying environmental factor involved in and incident to the gaining of energy and renewal of body substance by feeding is annulled. Considering the extremely constant environmental conditions in all respects under

which these starvation experiments were carried out, it seems reasonable to conclude that at least the major part of the residual individual variation in duration of life observed therein (expressed by coefficients of variation of about 20 per cent.) must represent inborn individual differences in the organization of the mechanism which we call *Drosophila,* due allowance being made for the *random* fluctuations of a magnitude indicated by the probable errors.

Sex Differences in Duration of Life under Complete Starvation

From Table III we have the following systems of sex difference in the means.

		Female mean—Male mean
Wild Type.	Density 5;	$45.03 - 44.50 = + .53 \pm .51$ (1.0)
	Density 50;	$46.94 - 42.21 = + 4.73 \pm .54$ (8.8)
	Density 100;	$50.89 - 44.30 = + 6.59 \pm .49$ (13.5)
Vestigial.	Density 5;	$48.72 - 43.97 = + 4.75 \pm .56$ (8.1)
	Density 50;	$50.31 - 44.07 = + 6.24 \pm .59$ (10.6)
	Density 100;	$52.47 - 44.74 = + 7.73 \pm .46$ (16.8)

It is evident that the *females have a longer mean duration of life than do the males under conditions of complete starvation.* The differences are all positive in sign, and five out of the six are eight or more times their probable errors. It seems safe to conclude that in general the sex differences are larger and steadier than could reasonably be supposed to have arisen from the fluctuations incident to random sampling alone. It is important to note that the direction of the sex differences (female longer-lived than male) is the same under starvation as under normal feeding. The significance of this result will be discussed more fully farther on.

Turning to the question of sex in relation to individual variation in duration of life we have to consider first the following system of differences in the standard deviations of Table III.

Female standard deviation—Male standard deviation

Wild type.	Density 5;	$8.91 - 8.77 = + .14 \pm .35$ (.4)
	Density 50;	$11.08 - 7.69 = + 3.39 \pm .38$ (8.9)
	Density 100;	$10.73 - 7.64 = + 3.09 \pm .35$ (8.8)
Vestigial.	Density 5;	$11.31 - 8.24 = + 3.07 \pm .39$ (7.9)
	Density 50;	$12.12 - 8.95 = + 3.17 \pm .41$ (7.7)
	Density 100;	$9.98 - 7.30 = + 2.68 \pm .32$ (8.4)

In all cases the *females exhibit a higher absolute variability in duration of life than do the males.* In all except the first comparison the differences are more than seven times their probable errors. In wild type flies at density five the sex difference in standard deviation is insignificant.

For the coefficient of variation we have the following system of sex differences:

Female Coefficient of Variation—Male Coefficient of Variation

Wild type.	Density 5;	$19.79 - 19.70 = + .09 \pm .83$ (0.1)
	Density 50;	$23.61 - 18.23 = + 5.38 \pm .87$ (6.2)
	Density 100;	$21.09 - 17.24 = + 3.85 \pm .75$ (5.1)
Vestigial.	Density 5;	$23.22 - 18.74 = + 4.48 \pm .87$ (5.1)
	Density 50;	$24.09 - 20.31 = + 3.78 \pm .90$ (4.2)
	Density 100;	$19.03 - 16.31 = + 2.72 \pm .68$ (4.0)

It is evident that in relative as well as absolute variability in duration of life under complete starvation the female is more variable than the male. All the differences are positive in sign, and all except the first may be safely regarded as larger than would probably arise from random sampling alone.

The Influence of Density of Population upon Duration of Life under Complete Starvation

In planning the present series of experiments it seemed desirable in the light of our earlier results (74) to employ at least three densities, one of which should be below the optimum density for fed flies, one at about the optimum and one well above. With this in mind densities of 5, 50 and 100 flies per one ounce bottles were chosen. The question was whether there would be dif-

ferences in duration of life under these three densities comparable relatively to the marked ones that we have found in fed flies. From Table I of (74) we have for fed flies:

Mean duration of life at density 4 = 29.64 ± .60
Mean duration of life at density 55 = 40.66 ± .53
Mean duration of life at density 105 = 24.94 ± .32

Thus from the low density to the optimal there is an increase in mean duration of life of 11.02 ± .80 days, or 37.2 per cent. above that at the low density. From the optimum density to a fairly high density of 105 flies per bottle there is a decrease in mean duration of life amounting to 15.72 ± .62 days, or 38.7 per cent. below that at the optimum. These are large differences, and our certainty as to their reality in fed flies has been firmly established by extensive experiments, a complete account of which will shortly be published.

The following system of differences derived from Tables III and IV shows the influence of density under complete starvation:

Mean duration of life at density 50—Mean duration of life at density 5

Wild type.	♂♂	; 42.21 — 44.50 = — 2.29 ± .48	hours	(4.8)
	♀♀	; 46.94 — 45.03 = + 1.91 ± .57	hours	(3.4)
	Both sexes	; 44.60 — 44.77 = — .17 ± .38	hours	(0.4)
Vestigial.	♂♂	; 44.07 — 43.97 = + .10 ± .48	hours	(0.2)
	♀♀	; 50.31 — 48.72 = + 1.59 ± .65	hours	(2.4)
	Both sexes	; 47.20 — 46.36 = + .84 ± .42	hours	(2.0)

Mean duration of life at density 50—Mean duration of life at density 100

Wild type.	♂♂	; 42.21 — 44.30 = — 2.09 ± .42	hours	(5.0)
	♀♀	; 46.94 — 50.89 = — 3.95 ± .59	hours	(6.7)
	Both sexes	; 44.60 — 47.69 = — 3.09 ± .38	hours	(8.1)
Vestigial.	♂♂	; 44.07 — 44.74 = — 0.67 ± .44	hours	(1.5)
	♀♀	; 50.31 — 52.47 = — 2.16 ± .60	hours	(3.6)
	Both sexes	; 47.20 — 48.51 = — 1.31 ± .39	hours	(3.4)

It is at once evident that density *per se* produces no such effect upon duration of life under complete starvation as it does under conditions of normal feeding. As between densities 5 and 50 the differences in the case of

the wild type flies are irregular as to sign, and near the border line as to significance. Taking both sexes together the difference is plainly not significant. Yet approximately the same difference in density has associated with it, in the case of fed flies, a difference in mean duration of life of about 37 per cent.

In the case of the vestigials the density difference from 5 to 50 has associated with it a *slightly* higher mean duration of life in the latter density, but the differences are too small to be statistically significant. The tabled differences might easily have arisen from chance fluctuations in sampling alone.

Turning to the density difference between 50 and 100 it is seen that the sign of the duration of life differences is in all cases negative. In other words, under conditions of complete starvation both wild type and vestigial flies have a mean duration of life of four to five per cent. longer at density 100 than at density 50. This is directly opposite to what is found in fed flies, as has already been pointed out. The differences are also *relatively* smaller. But in the case of the wild type flies they are absolutely large enough to be probably significant statistically. In the case of the vestigials, the differences are less probably significant.

Altogether it is apparent from these results that a considerable part of the differences in duration of life associated with differences in density of population in the case of fed flies *must be directly connected with feeding,* because they become very much smaller relatively under conditions of complete starvation. There seems no escape from this conclusion. It is important in two ways. In the first place it suggests at least a partial explanation for some of the puzzling results which we have demonstrated in regard to the effect of density upon duration of life; and in the second place it points the way to new experimental points of attack upon the density problem.

There is a slight, but still fairly definite suggestion of a tendency for duration of life under conditions of com-

plete starvation to *increase* as density increases, up to a density of 100 flies per one ounce bottle at least. This is especially marked in the case of vestigials. The determination of what this means relative to the general problem of density effects must await further experimentation, but the probable fact is worthy of some emphasis now, lest it be overlooked in future discussions of the density problem.

There appears to be very little effect of density of population upon *variation* in duration of life, under conditions of complete starvation.

The Form of the Life Curve under Starvation

We come now to the consideration of the chief problem set at the outstart of this investigation. Do wild type and vestigial flies show the same form of life curve under conditions of starvation and of feeding? To get light on this point it is necessary to put the two cases on a basis of centile age instead of absolute age, according to the plan described in earlier studies in this series (*cf.* 61, 63 and 66). This is done in Figs. 5 and 6. In Fig. 5 the centile age survivorship curve for Line 107 wild type males under conditions of normal feeding (66, Table VI, p. 79) is compared with the centile age survivorship curve for wild type males of the same line under starvation conditions and initial density 50. In Fig. 6 the centile age survivorship curve for vestigial males under normal feeding (66, Table VI) is compared with the corresponding curve for vestigial males under starvation conditions and initial density 100. It is not thought necessary to compare the curve for females or for other densities, because nothing different in principle would be brought out. Also it has not been deemed necessary for present purposes to graduate the starvation curves. They are sufficiently smooth as they are to show clearly the essential point.

An examination of these diagrams makes it clear that so far as wild type flies are concerned the form of the

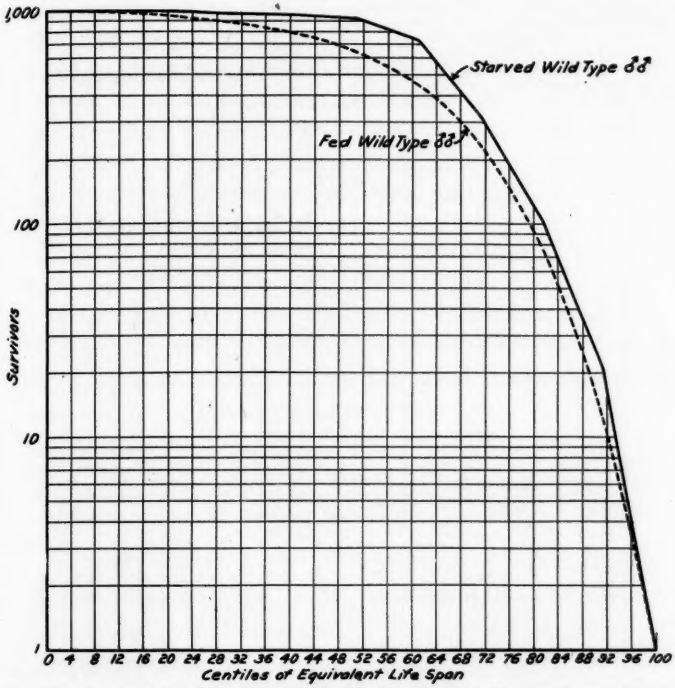
1000
100
10
1
Survivors
Starved Wild Type ♂♂
Fed Wild Type ♂♂
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 88 92 96 100
Centiles of Equivalent Life Span

FIG. 5. Comparing the survivorship curves on a centile age base of (*a*) starved wild type ♂ ♂ (solid line) and (*b*) fed wild type ♂ ♂ (broken line).

life curve is substantially the same whether the individuals are fed or starved. The specific death rates are somewhat *lower* during the first half of the equivalent life span in the case of the starved flies, but at equivalent ages after about centile 60 the rates are practically identical. And in the earlier half of the span the differences are after all insignificantly small, in all probability.

The case is very different with the vestigial flies (Fig. 6). There the starvation curve is, as has already been seen, essentially similar in shape to the wild type starvation curve, which in turn has the normal form, common to man, *Drosophila* and other forms under normal feeding conditions. But this is quite different from the form

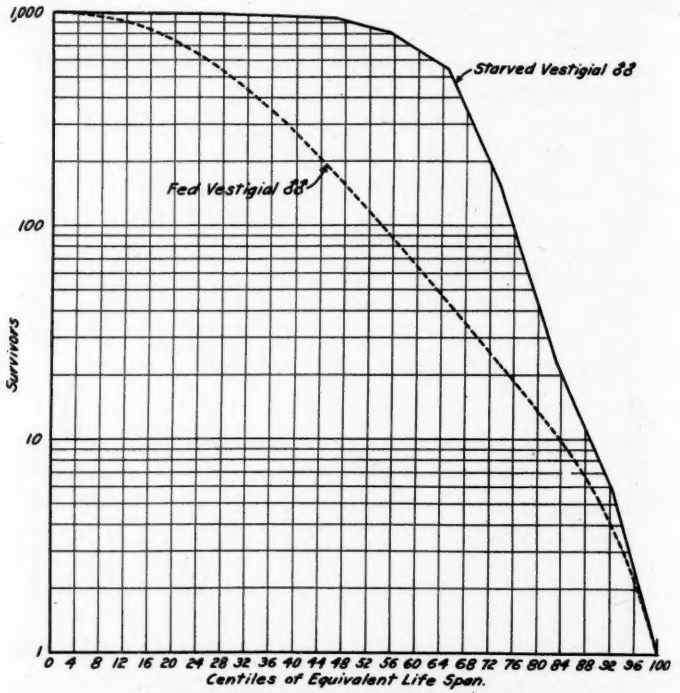
1,000
100
10
1
Survivors
Starved Vestigial ♂♂
Fed Vestigial ♂♂
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 88 92 96 100
Centiles of Equivalent Life Span.

FIG. 6. Like Fig. 5, but for vestigial ♂ ♂.

of the survivorship curve upon a centile age base of vestigials under conditions of normal feeding. This latter curve, as we have already pointed out in (66), approaches closely to the straight diagonal on an arithlog grid which would be given by absolutely uniform specific death rates throughout life.

So then it appears that while it would be possible to make a very close approximation to the normal life table of normally fed flies of *wild type* by observing *only* the mortality of wild-type flies under complete starvation, it would be wholly impossible to do this for *vestigial* flies. This suggests that what we call normal laboratory feeding conditions do not permit vestigial flies to realize their full innate potentialities in respect of duration of life. It

also suggests that just as vestigials live quite as long as wild-type flies under complete starvation, it might be possible to devise a system of *feeding* vestigials which would enable them to live just as long as fed wild-type flies. Preliminary experiments indicate clearly that this is the fact.

The genetic significance of the facts brought out in this section will be discussed in the next section.

Discussion

From the data given by Lutz (13) regarding the duration of life of *Drosophila* (wild type) given water, but no food, we have calculated the following constants for comparison with those of this paper:

Mean duration of life =	(♂♂) 66.31 ± .60 hours;	(♀♀) 68.61 ± .62 hours
Standard deviation =	(♂♂) 9.51 ± .42 hours;	(♀♀) 10.17 ± .44 hours
Coefficient of variation =	(♂♂) 14.35 ± .65	(♀♀) 14.83 ± .65

Comparing these values with those of Table III, *supra,* it is evident that the addition of drinking water has considerably increased the mean duration of life under complete absence of food. This result is probably due to two things. First, animals generally endure starvation better if they have water to drink than if they lack it, even though the atmosphere in the latter case is saturated with moisture, so that no desiccation occurs. Second, it is probable that the water furnished contained minute amounts of material in solution, and bacteria and yeasts washed off the bodies of the flies themselves which served in some measure as food.

Lutz's experience confirms ours in respect of the reduced *variation* in duration of life under conditions of starvation. The standard deviations are of about the same area of magnitude as ours, while, of course, the coefficients of variation are lower, owing to the larger means.

The most striking general result of the present study is the finding that while under normal feeding conditions

wild-type flies live on the average about three times as long as vestigials, nevertheless when placed under conditions of complete starvation the vestigials live as long as the wild type. This fact is of great importance from the point of view of genetics. It is a specific example of the general principle that the somatic expression of any genetic factor in any particular case is in part a function of the general environmental level which prevails in that case. It has been demonstrated (57, 62) that *under the standard feeding conditions for laboratory-bred Drosophila* the gene for vestigial has as a part of its somatic expression a very considerably reduced duration of life as compared with the wild type. There are few cleaner cut cases of Mendelian segregation to be found in the whole literature of genetics than that upon which the above statement is made. Yet the present study shows that the whole of that part of the somatic expression of the vestigial gene which is differential in respect of duration of life disappears under another system of "feeding" wild-type and vestigial flies (namely, complete starvation). This fact does not in the least invalidate the earlier results cited on the inheritance of duration of life. Those results are *facts* just as much as the present ones. It merely emphasizes once more, in a rather striking way, the extraordinary caution which is always necessary in interpreting the results of genetic experiments. It also, of course, points the way to new and very promising lines of further experimental attack upon the problem of duration of life.

The general genetic principle enunciated above has been recognized, formally at least, by many workers. A case which in some important respects of principle closely parallels the one here presented has lately been described by Metz (77), having to do with the effect of temperature on the expression of the mutant gene "bent," in *Drosophila*.

Pursuing this same subject of the influence of environmental conditions upon the expression of inborn, genetic

characteristics a little further, it is of interest to note that the same kind of difference in mean duration of life which is observed between the two sexes under conditions of normal feeding is also found under starvation. We interpret this result to mean that the innate constitutional differences which distinguish the two sexes are of a much more fundamental character than those consequent upon the presence or absence of the mutant gene vestigial. Those differences in innate constitutional make-up or organization which make a female *Drosophila* live longer on the average than a male are too deep-seated to be upset by the most extreme differences in feeding (full feeding versus complete starvation).

Summary

In this paper are given the results of the determination of duration of life in each of 3,632 individuals of *Drosophila,* under conditions of complete starvation. The chief results are:

1. Under complete starvation the mean duration of life and the form of the life curve are practically the same in vestigial as in normal wild-type flies, though under conditions of full feeding the wild-type flies live roughly three times as long as the vestigials.

2. There is a much reduced relative variability in duration of life under conditions of complete starvation than in conditions of full feeding.

3. The normal relation between the sexes in respect of mean duration of life (females longer-lived than males) observed under full feeding, is preserved under conditions of complete starvation.

4. Females are more variable than males, both absolutely and relatively, in duration of life under complete starvation.

5. The relation of duration of life to density of population is quite different under complete starvation from what it is under full feeding.

The genetic significance of these results is discussed.

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THE PHYSICO-CHEMICAL CONDITIONS OF MORPHOGENESIS*

RALPH S. LILLIE

THE attainment of definite or final form in animals and plants (either of the whole organism or of its special parts) usually comes at the end of a more or less prolonged sequence of growth processes, accompanied by a progressive and orderly deposition of permanent structure. This emergence of definite structure in the growing mass, with associated definite function, is the process usually called "differentiation." The essential problem of morphogenesis is the problem of why the form and structure thus attained should be constant for a given species, *i.e.*, should recur constantly in successive generations. This problem, as thus defined, becomes almost indistinguishable from the problem of heredity, the chief data of which are structural characters, or physiological characters based on structural characters. The physiological study of this problem is still in an early stage. We know that the adult structural characters are correlated in a definite manner with the structural characters of the germ cells. In particular, the relation between the number, structure and behavior of the chromosomes and the later appearance of somatic characters has been investigated lately in great detail and with a striking degree of success. We can now predict with a fair degree of statistical accuracy what special structural characters will emerge at the end of the morphogenetic sequence when two germ-cells of a given Mendelian constitution unite in fertilization. Such possibility of prediction shows that the series of formative processes is dependent in its finer details upon the nature of the initial combination occurring in maturation and fertilization. In itself, how-

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ever, it tells us little concerning the detailed physiological nature of these processes. The latter problem is one for physiology.

Briefly the physiological problem may be defined as the problem of the nature of the physico-chemical processes concerned in the formation of organic structure. In our study of this problem we omit all consideration of final causes and assume that at every stage the efficient causes are physical. The constancies observed in external nature are physical constancies and have their root in physical conditions. It is clear that the existence and perpetuation of organisms depend upon processes whose essential features are constancy, determinability and order. Stability of process is the primary requisite for constancy of outcome in any kind of development. Morphogenetic processes exhibit the highest form of order. As Santayana puts it, "Only order can beget a world or evoke a sensation."

That natural processes under defined conditions pursue their course with exactitude is simply a fact of observation. This, which we recognize as true of simple physical sequences, is equally true of the complex processes of growth and morphogenesis which begin with fertilization and end with the emergence of the imago from the cocoon. Such sequences resemble simple physical sequences in the one fundamental respect, namely, their determinability. We know of many simple and regular morphogenetic sequences in inorganic nature, for example, the sequence by which a supersaturated solution (not inaptly called a "mother liquor") yields a crop of crystals of definite form when sown with solid fragments of the dissolved compound. Here we are bringing into comparison (or at least into simultaneous consideration) processes which have in common but one feature, namely, the production of a physical complex, having a definite structure and form, as the end-result of an accumulation or accretion which starts from a minute germ or center structurally much simpler than the final product. Both instances ex-

emplify what is called "germ-action," a term transferred to physical science from biological observation. It is especially to be noted that the nature of the formative process is determined both by the physico-chemical constitution of the original center and by the conditions in the surroundings; *i.e.*, two sets of factors, internal (those initially present in the germ) and external, are concerned. Under the same conditions in the surroundings two living germs or two crystals may carry through entirely different sequences of morphogenetic processes. A solution supersaturated with more than one compound separates out crystals of the compound which is introduced in solid form; similarly, a yeast or mould introduced into a culture medium forms a center of construction of organized structure of a specific kind. Both crystal and organism are accretions, their materials are collected from the surroundings; both attain definite form, structure and physical properties at the end of a sequence, more or less complex, of form-determining processes. This fundamental resemblance can not fail to be significant, however great may be the differences in the details of the two sequences.

It will be understood that in thus reverting to the time-honored comparison of inorganic crystallization with organic morphogenesis, I am not implying any complete identity of the two processes. What I do imply, however, is that the conditions which render possible definite form-determination in the inorganic system are also an indispensable part of the conditions underlying the specific form-determination in the living system. Without constancy in the finer elements of organic structure, constancy in the structure of the whole organism would hardly be possible. Underlying the constancy of organic form we must assume the constancy of sub-organic form. This means constancy of the antecedent form-determining conditions. The synthesis by which the adult organic form is constructed with such regularity is based upon the synthesis of those specific chemical compounds which

compose its finer structural elements. These compounds as they separate in solid form to form the structural substratum of protoplasm adopt a finer structure which we have every reason to believe is in the last analysis crystalline. It is known that in many cases the separation of proteins (the chief structure-forming compounds of protoplasm) from solution is in crystalline form *e.g.*, fibrinogen solutions deposit crystals of fibrin when they clot; the internal structure of many gels has been shown to be crystalline; and crystals are formed in the "salting-out" of many proteins. In general, underlying and determining crystalline growth is a process of surface deposition which is in the nature of an adsorption; in adsorption the adsorbed molecules adopt regular orientation at the surfaces, as the recent work of Langmuir, Harkins and Adam has shown. The regularity of the crystal-form is determined by this regularity of surface orientation. All the indications are that organic growth also proceeds by the deposition of new material at the surfaces of structures already laid down. This process implies the formation of new structure; and the special nature of this structure is determined by the chemical composition of the material which is thus deposited.

Crystalline structure is the visible evidence of the orderly arrangement of the atoms in molecules and of the molecules in molar masses. We know from the results of the X-ray analysis of crystal structure that the atoms in solids have fixed positions relatively to one another, and that these positions depend on chemical constitution. The whole structure formed by the cohering molecules has the regularity of an accurately ruled grating; this structural regularity is what makes possible this mode of physical analysis. The atomic groups which, when detached from one another (*e.g.*, in solution), we call molecules, have in crystals a close-packed arrangement with the axes parallel and the interatomic distances and angles constant and measurable. The definite form adopted by the molar structure—the crystal—is the expression of

this regularity in the structure of the individual molecules and in their manner of coherence. The existence of cleavage planes, as well as of other constant properties, such as tenacity, elasticity, refractive index, etc., is most readily understood on this conception. Cleavage planes, for example, are the planes along which the adjacent layers of molecules are most readily separated from one another. In fusion or solution the molecules lose regular orientation as they lose coherence; the form characters of the aggregate are then lost. The original form is regained when the range of molecular movement is reduced by lowering the temperature, or when the solvent is removed. The morphogenetic process by which the crystal is formed is thus characteristically reversible; the same is true of certain types of organic morphogenesis. Recently the crystalline structure of relatively complex organic compounds like naphthalene has been investigated with success by Sir William Bragg, and the results are in close conformity with the conception of molecular structure derived from a consideration of purely chemical properties. Astbury's recent studies of the X-ray structure of tartaric acid bear out completely Pasteur's conception of the asymmetric arrangement of the atoms; *i.e.*, there is direct optical evidence of the existence of two atomic arrangements, one the mirror-image of the other.[1] Two conclusions follow, both applicable to our biological problem, first, that the finest elements in a solid structure have a regularity of a definite repetitive kind, and second, that in complex organic compounds the special structure adopted is determined by the stereochemical constitution of the molecules.

The arrangement of the molecules in solid structures is determined by the specific chemical constitution of the molecules, and this is determined by the dimensions, valences, force-fields and other characteristics of the combined atoms. The characteristics of each atom, accord-

[1] *Cf.* W. T. Astbury: "Analysis of crystal structure by X-rays." *Science Progress*, 1923, Vol. 17, p. 386.

ing to the electron theory, are determined by the relative numbers, arrangements and motions of the electrons grouped about the central positive nucleus. From crystalline or molar structure to molecular structure, and from molecular to atomic structure, and from atomic structure to the properties and motions of the electrons, these characteristic relations of interdependence extend. These relations are asymmetrical in the logical sense, *i.e.*, the characteristics of each larger product of synthesis are based upon or presuppose those of the smaller; but the smaller exist and have their properties independently of the larger. So that while in analysis we have a convergence to simplicity (to use Whitehead's phrase[2]), in synthesis unlimited possibilities of diversity present themselves. Although many of these possibilities have been realized in nature, no limit can be assigned to the synthetic process, and evolution is still in active progress.

Professor Niels Bohr is reported to have said in his recent Yale lectures that a true interpretation of atomic structure opens the way to a reversal of the usual order of scientific explanation. Our study of the universe may begin with the smallest particles of matter and work upward, *i.e.*, synthetically, instead of downward (as hitherto) from large masses to the minute. Proceeding in such a manner we might retrace in outline the process of creative evolution by which the present status of the physical universe has been reached. We should see how the synthesis of molecules from atoms has followed the synthesis of atoms from protons and electrons. The synthesis of molar or solid structure (which is essentially crystalline structure) has followed as the next step in synthesis. Finally, we should come to the synthesis of special interest to biologists, that by which organic structure with its correlative physiological activity has arisen from a combination of inorganic materials and energies.

The subject of our present discussion is morphogenesis in the biological field. Morphogenesis, the production of

[2] "The Concept of Nature."

definite form and structure, is a constant expression or result of vital activity, characteristic for each species. The materials that form the permanent structure of any organism are either synthesized by the organism or collected from the surroundings; in either case they are deposited in solid form in definite situations during the period of growth. The order and locus of their deposition are determined by uniformly acting factors, partly physical, partly physiological. Summarizing the foregoing brief analysis, we may say that the fundamental or primary morphogenetic factors are of a general or nonspecific physical kind, of the same nature as those underlying crystallization, and that superposed on these simpler physical factors are more complex secondary or "physiological" factors which are specific and vary from organism to organism.

Let us now briefly consider the nature of the more specifically vital or physiological factors of morphogenesis. The chief questions are the following: (1) What is the special physical and chemical nature of the material which forms the living or organized structures? and (2) why does it assume the form which we observe in each special case? These problems may be regarded as the special problems of morphogenesis as distinguished from the other general problems of physiology from which, however, they can not be separated. In a broad sense these problems are the same in all organisms, although in higher organisms the simple fundamental conditions are likely to be obscured by special complications which from the physical point of view are mainly incidental. The living material is plastic and may adopt almost any form consistent with its fundamental requirements, access of nutrients and oxygen and removal of waste metabolic products.

In seeking factors common to all cases of morphogenesis we may therefore find it more instructive to consider first the simpler organisms or single cells. Why do single cells under favorable conditions of nutrition and

oxygen-supply attain definite form and dimensions? In higher organisms cellular form and dimensions are constant within certain well-defined limits of variation. The nuclear-plasma relations are also constant; the same is true of the structure and mode of deposition of the finer structural elements, such as the fibrils in muscle cells. Bacteria, yeast cells and protozoa growing in culture media also attain constant form, structure and dimensions, resembling in this respect the cells of higher organisms. Such general relations are usually interpreted as indicating that when in growth the ratio of surface to volume is reduced below a certain volume, division ensues. The surface-volume ratio thus passes through a rhythmical variation about a certain mean, decreasing at the interdivisional periods and increasing at division. Under normal conditions, this mean is maintained with constancy, and this constancy ensures the constancy of the cellular dimensions. But evidently underlying this regulative process are the metabolic reactions of the protoplasm, which determine the special nature of the added material. These must have a similar cyclical character.

Robertson's recent researches[3] render it probable that in protozoa growth is controlled by the production within the nucleus of a substance which has an autocatalytic relation to certain metabolic reactions essential to growth. The distribution of this substance between nucleus and cytoplasm and between cytoplasm and external medium occurs periodically at times of division. This is the time at which nuclear and plasma membranes undergo increase of permeability. The observed sequence, growth to a certain size, division and resumption of growth to the same size, may thus in a measure be understood, division corresponding to a stage when a certain accumulation of constituents has occurred within the cell. The concentration-relations of such a substance will be deter-

[3] *Cf.* Robertson: "The Chemical Basis of Growth and Senescence," Philadelphia, 1923.

mined both by the nuclear-plasma volume-ratio and by the ratio of the general cytoplasmic surface to the volume enclosed (or specific surface of the cell); in this way, a size-regulating mechanism has been evolved, depending ultimately on conditions of chemical equilibrium.

Regulation of cell-size is, however, only one elementary feature of morphogenetic processes. The regulation of cell-structure is of more immediate interest. We have to account for the constant form-characters of the structures which are laid down within the cell as it grows and develops. Now, broadly speaking, when we consider the relations, already briefly sketched, between molecular structure and the molar structure of materials in the solid state, we can see that the solid metabolic products appearing in the cell must, as they are deposited, tend to form structure of a constant kind. The reason why these structures are specific and constant for each cell must, therefore, be intimately related to the fact that their chemical composition is also specific and constant.

The relation of proteins to specificity has often been discussed. There is a correspondence between the diversity of native proteins and the diversity of native organic species, although proteins are more numerous than species, since most organisms have a variety of specific proteins. When corresponding proteins from two species of animals are chemically similar (as shown by precipitin or anaphylaxis tests), we find also that the species are structurally and physiologically similar. Proteins from nearly related animals, when separated from solution under conditions favorable to crystallization, form aggregates which are structurally similar; this has been shown most clearly by Reichert and Brown in the case of hemoglobins. What is true for these proteins must be true for others. Similarly constituted compounds form similar structural aggregates. If this is the case *in vitro* we may assume that it is also the case when the compounds are laid down within or between the cells to form organic structure. Chemical similarity determines struc-

tural similarity, and this secondarily determines physiological similarity. It is true that many other compounds besides proteins are present in protoplasm, and that the nature and proportions and concentrations of these are also constant for a particular species of cell; these compounds also play a part in determining structure. Yet the peculiar physical properties of proteins seem to determine what is specific in protoplasmic structure, *i.e.*, why one species of cell differs characteristically from another. For example, that the plasma membrane of one species of blood corpuscle is structurally different from that of another is shown by the differences in physical properties (iso-electric points, permeability, resistance to hemolysis, etc.); and that there is a corresponding difference in the structural proteins is shown by the existence of specific hemolysins.

How are the proteins formed within the cell? And what determines the peculiar specificity which they show? Why should one species of cell form constantly proteins of a definite stereochemical configuration? These problems are not yet solved. We merely know that from the same mixture of amino-acids one cell synthesizes one set of proteins, and another cell another set. In this process we have again a form of germ action. Something analogous to accretion occurs; external compounds are attracted and enter the cell; but this accretion is associated with a characteristic sorting activity, combined with dehydrolytic condensation, as a result of which the amino-acids are linked in definite order. Typically, this order corresponds to that characteristic of the structural proteins already present. For example, we observe that the fibrils of an exercised muscle cell increase in size and number. This process is not identical with crystallization, but it has its affinities with this process, *i.e.*, underlying it are processes of the same nature as those determining crystallization. The amino-acids are not only attracted in some manner to the structure already present, but they are dehydrolytically condensed to form the

same structural proteins. Of the chemical details of this process we are still ignorant. Apparently oxidations are necessary, especially of carbohydrate. We know that a separation and rearrangement of amino-acids occurs within the cell in many cases, *e.g.*, those in which protozoa or bacteria grow in a medium containing protein but no free amino-acids. The cells, however, contain proteases, so that conditions for hydrolysis are present. What is not yet understood are the conditions determining the specific rearrangement.

Yet these conditions must be understood if we are to understand growth and morphogenesis in any fundamental sense. Some light is thrown on the problem by the facts of immunology. We know that the introduction of foreign proteins into the circulation of a higher animal results in the formation, by the cells of the animal, of compounds (antibodies) which are similar or complementary in their configuration to those introduced. This seems to indicate that in the presence of one protein the formation of another of similar structure is promoted or catalyzed. It is true that anti-bodies are not identical with antigens; they are, however, structurally closely related, so that close adhesion and union occur when both are present. The essential fact is that there is a correspondence in configuration between the introduced compound and the compound which is synthesized by the living protoplasm under its influence. Similarly, in the growing muscle cell the proteins already present in some manner enable metabolism to produce identical protein and hence identical structure. The precise physico-chemical conditions of this reduplication are unknown. We may leave the problem in this stage, the stage of definition. Its solution can only follow further study and experiment.

Two years ago at a meeting of this society Professor Muller called attention to the bearing of the d'Herelle phenomenon on a related problem, the problem of the multiplication of genes.[4] In this phenomenon, first ob-

[4] H. J. Muller: AMER. NATURALIST, 1922, Vol. 56, p. 32.

served by d'Herelle in the bacillus of dysentery, the characteristic feature is the continued reproduction of a substance, specifically lethal to the organism, in response to the introduction of a small quantity of the same substance into the culture medium. Similar phenomena are known in other organisms, for example, the multiplication of contagious viruses in certain plant diseases, *e.g.*, mosaic disease of tobacco or infectious chlorosis of Malvaceae;[5] the growing parts produce the specific toxic compound, which is identified by its lethal action on other growing parts. The synthesis of a new specific compound, ordinarily not present in the protoplasm, is promoted or autocatalyzed by its own presence. These lethal compounds, as well as the bacteriophage in the d'Herelle phenomenon, multiply only during the process of cell-multiplication, *i.e.*, in association with the other specific syntheses of the cell-protoplasm. The lethal character is incidental, like the lethal character of certain genes; if the compounds were not lethal they would not be detected and their specific nature established. What is of interest is that their presence forms the condition for the metabolic synthesis of more material of the same kind. This property is probably universal in protoplasm, and apparently constitutes the basis of its characteristic self-multiplying property or power of growth.

Finally, we come to a consideration of the conditions determining the special and often complex form of multicellular organisms. Here the factors are correspondingly complex and our knowledge is in an early stage. It is clear, however, as d'Arcy Thompson has recently pointed out,[6] that the form adopted by any growing animal or plant is an index of the relative rates of growth in different regions. The problem resolves itself into the problem of the conditions determining the local differences in rate of growth. Only when we understand the nature of

[5] *Cf.* the recent review by C. E. Simon, "The filterable viruses," *Physiol. Reviews*, 1923, Vol. 3, p. 483, for further details and references.

[6] "Growth and Form."

growth and the factors determining its rate shall we be in a position to understand why each organism assumes a definite form as it develops. Certain generalizations drawn from the observation of morphogenetic processes have a fundamental significance in relation to this problem; for example, the law of anteroposterior development, which expresses a generally observed tendency for the anterior regions of bilateral animals to grow and differentiate more rapidly than the posterior regions. Child has shown that this gradient in the rate of growth is correlated with a gradient of susceptibility to poisons and with a gradient of oxygen consumption and of carbon dioxide production. There is also a gradient of electrical potential. The secondary axes of the growing organism appear to have similar properties. In general, the presence of growing apices or growing zones is a highly characteristic feature of multicellular organisms. The actively growing regions are definitely localized with reference to the chief axes; that these regions correspond with actively metabolizing regions is clear, apart from the evidence cited above, since growth is an expression of constructive metabolism. An additional fact of general significance is that such regions have typically a characteristic physiological influence on adjoining regions, repressing or inhibiting the growth of the latter. The whole structure thus exhibits a physiological polarity of a special kind; morphogenetic polarity is one expression of this polarity, the general conditions of which remain to be considered.

The physico-chemical conditions that determine this physiological or metabolic polarity are evidently of critical importance in the morphogenetic process. A combination of structural and active (*i.e.*, physiological) factors must be assumed. The first question to decide is whether this polarity is fixed by internal organization, or dependent wholly or in part on external conditions. Many attempts have been made to alter or reverse polarity by experimental means. Apparently in higher animals the

internal or constitutional factors are usually too fixed to admit of such change. In lower organisms, however, experimental reversal of polarity has been accomplished in a number of instances. These cases appear to be the most significant from the point of view of the physiology of morphogenesis. We know that the direction of growth, especially in plants, is readily influenced by external agencies, chemical, mechanical and electrical. In cut hydroid stems, differences of oxygen supply may determine morphogenetic polarity, as when the free end develops the hydranth, the buried end the stolon. Recently Lund has shown that the polarity of these stems may be controlled by the electric current;[7] other recent evidence from both animals and plants has also illustrated the directive influence of the electric current on growth. And since organic polarity indicates metabolic or chemical polarity, we are led to consider the general conditions under which chemical polarity is exhibited in other natural systems.

Broadly speaking, the most general conditions determining chemical polarity—by which term we mean reciprocality and mutual interdependence of the chemical reactions in two regions of a reaction-system—are electrical. The difference between the chemical processes at the anode and at the cathode of a battery or electrolytic cell appears to present the closest inorganic parallel to the difference between the chemical processes at the growing and the quiescent regions of an organic structure showing polarity of growth. In the regenerating hydroid stem the hydranth-forming region is electrically negative to other regions, as Mathews and Lund have shown; and a similar polarity has been shown to exist in other cases. This fact is to be correlated with the fact that morphogenetic polarity may be reversed in certain cases by the use of electric currents of appropriate intensity.

The influence of electric currents on growth processes is one manifestation of the general electrical sensitivity

[7] Lund: *Journ. Exper. Zool.*, 1921, Vol. 34, p. 471.

of living matter. This sensitivity is based on its partitioned or film-pervaded structure, *i.e.*, on the presence of polarizable (*i.e.*, semi-permeable) partitions or membranes surrounding and penetrating the living protoplasm. This condition is a feature of its emulsion-like or alveolar structure. Changes in the electrical polarization of these partitions occasion chemical reactions when an electric current passes through such a system. In a general sense it is not remarkable that electric currents should influence growth, since they influence so many other chemical processes in living protoplasm; this is best shown by the universal fact of electrical stimulation. Electrical stimulation has long been known to be a polar phenomenon, and the same is true for the directive influence of the electric current on growth.

The direction of the bioelectric currents ("growth currents") in growing organisms has a definite relation to the regions of rapid growth. Most observers agree that the rapidly growing regions are electrically negative relatively to slowly growing regions, *i.e.*, are the regions where the current (positive stream) of the bioelectric circuit enters the protoplasm from the surroundings. Now, in general, in any arrangement of two conductors separated by a polarizable surface and traversed by a current (*e.g.*, a metal-electrolyte combination) the region where the positive stream enters the chemically reactive phase is the seat of chemical reactions of an oxidative kind; this is illustrated in the oxidations occurring at the anode of a battery or electrolytic cell. If the cell-surface is comparable with an electrode surface, we should expect that the region where the positive stream enters the protoplasm from the surroundings would similarly be the region where oxidations are promoted. It seems probable that this is the general physico-chemical explanation of the association of electrical negativity with regions of active growth. The "negative" regions are those where the positive stream of the bioelectric circuit enters the protoplasm. Such regions are also the regions of most

active oxygen consumption. In Lund's experiments on the reversal of polarity in Obelia by the constant current, he found that the formation of hydranths from the cut hydroid stems was promoted at the ends facing the anode, *i.e.*, where the positive stream of the external current enters the protoplasm from the surroundings. Since in regeneration under the normal conditions the region of hydranth-formation is the region where the normal bioelectric current has this direction, it seems clear that the reversal of morphogenetic polarity corresponds to a reversal of the direction in which electric currents traverse the living system.

If the electric current promotes oxidations where it enters the living system from the surroundings, it should also promote processes dependent on oxidations, including synthesis and growth. The relation of oxidations to protoplasmic syntheses is not understood in detail. Some of the general physico-chemical conditions of intracellular oxidations appear, however, to be now well established. We know from the facts of electrical stimulation and narcosis and from the general dependence of metabolic reactions on protoplasmic structure that the chemical activity of living matter is a function of its polyphasic composition, *i.e.*, of the presence of phases or structural elements bounded by electrically polarizable surfaces or partitions. There are many indications that the syntheses underlying growth-processes occur at the surfaces of the protoplasmic phases; this is, in fact, implied by the susceptibility of these processes to electrical control. Nernst and others have shown that the electric current affects living protoplasm through its polarizing action, a fact indicating that the electrically induced reactions occur at the surfaces where polarization is altered by the current. The same is true of the reactions at metallic electrodes, which are also surface reactions. The narcotizability of growth processes by surface-active compounds is further evidence of this condition, since this form of narcosis appears to be dependent on the dis-

placement of oxidizable compounds from the protoplasmic surfaces, as indicated especially by Warburg's recent work.[8]

Warburg has shown that amino-acids undergo rapid catalytic oxidation at the surface of animal charcoal, although they are highly stable compounds in homogeneous solution. This fact is highly important from the present point of view, since it shows that the reactivity of these compounds, when adsorbed, *i.e.*, when under the influence of electrically polarized surfaces, is very different from their reactivity in homogeneous solution. If these compounds are readily oxidized under these conditions, it may be assumed that the readiness with which they enter into other reactions is also increased. It is true that the formation of proteins from amino acids is not an oxidative but a dehydrolytic process, but it appears to be dependent in some manner upon the intracellular oxidations. Just why oxidations, especially of carbohydrate, should be so essential to the dehydrolytic condensations underlying growth is not clear; a suggestive fact is that the action of dehydrating agents (hypertonic sea-water) in artificial parthenogenesis is similarly dependent on oxidations. Possibly the protoplasmic dehydrations depend on alternate oxidations and reductions; but this again is a problem which awaits solution. Specific synthesis requires a specific rearrangement of amino-acids; presumably the energy of the work thus performed comes from oxidation, but many features of the problem remain obscure.

The hypothesis that synthesis and deposition at the protoplasmic surfaces is the essential underlying condition of organic growth processes explains in a general way the relation of the electric current to growth; it also explains the interferences which result when highly adsorbable indifferent compounds (narcotics) are introduced into the protoplasmic system. The detailed nature of the formative process depends on other conditions,

[8] *Cf.* Warburg, Biochem. Zeitschr., 1921, Vol. 119, p. 134.

partly reviewed above; in addition to chemical specificity, other factors enter, the nature of which can only be surmised at present. Analogies may be pointed out between growth processes and certain forms of electro-deposition or electrosynthesis. In the electro-deposition of metals at cathodes, characteristic structure is formed which is often branching, vesicular or in other respects similar to organic structure. The precise structure of such deposits is known to depend on various factors, such as density of current, presence of other compounds in solution, *e.g.*, surface-active compounds, or hydrogen ion concentration. It is possible that the modifiability of morphogenetic processes by hormone influence, chromosomal constitution or other forms of chemical influence may depend on conditions which are related in their fundamental aspects to physical conditions of this kind—however widely the protoplasmic processes may differ in their chemical details. The degree to which such conceptions can be applied with advantage in the physiological study of morphogenesis can be determined only by future investigation.

MODIFICATION OF DEVELOPMENT IN RELATION TO DIFFERENTIAL SUSCEPTIBILITY[1]

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ACCORDING to the older preformistic theories of heredity, there was, strictly speaking, no problem of development as distinct from the problem of heredity. The course of development was predetermined by the hereditary mechanism, at least for the earlier stages, and modifications were possible only as this mechanism was modified. The older epigenetic theories assumed the action of environmental factors, but were unable to present demonstrative evidence in support of their conclusions. The theories of heredity now current, however, do not include a theory of development. They tell us that every cell inherits the whole germ plasm, but they do not tell us how the cells of the multicellular organism become different in the course of development. If we suppose that these differences result from distribution to different cells of substances regionally localized in the cytoplasm of the egg, we have still to account for the origin and distribution of the substances in the egg cytoplasm. If these are determined by reaction between the genes and the cytoplasm in the egg, we should expect, since every cell inherits the whole germ plasm, that many, if not all, cells would come in course of time to resemble the egg more or less closely, even though they were more or less different as regards their cytoplasm at the time of their origin. The only possible view seems to be that regional cytoplasmic differences arise in relation to factors external to the protoplasm concerned. If this be true, the

[1] Second paper of symposium of the American Society of Zoologists in conjunction with the American Society of Naturalists and the Botanical Society of America on "Morphogenesis." Read at Cincinnati, December 29, 1923.

problem of the origin, nature and significance of these cytoplasmic differences constitutes a problem of development distinct from the problem of heredity. The present paper is concerned with certain aspects of this developmental problem.

Physiological polarity and symmetry are expressions of factors in some way concerned in development with the spatial localization and order and the chronological sequence in appearance of specialized structures and functions characteristic of the individual, that is, with the general features of individual pattern. Since they are of such general significance and appear in protoplasms of widely different constitution, they must be in large measure independent of specific protoplasmic constitution. That polarity at least has been so regarded is indicated by the fact that it has often been assumed to be a fundamental property of living protoplasm.

The assumption has often been made that the physiological polarity and symmetry of organisms are based on a structure and orientation of molecular or molar particles, either analogous to that of the crystal or of unknown nature, and biological literature contains various attempts to interpret phenomena of individual development and reconstitution in such terms. These hypotheses are open to various objections: First, studies with polarized light give evidence of such structure only in certain specialized parts, not in protoplasm in general; second, if such structure exists in protoplasm we should expect it to be extremely stable and not readily altered by external conditions, but polarity and symmetry, at least in the simpler organisms, can be altered in many ways, or even obliterated and determined anew by factors which we can not readily conceive as altering such a fundamental structure; third, the results of reconstitution of isolated pieces, particularly the development of partial and biaxial structures, can not be interpreted in terms of these hypotheses without special assumptions for the special cases; fourth, crystalline structure disappears when

chemical reaction takes place and it is difficult to conceive how such a structure of the magnitude necessary as a basis for physiological polarity and symmetry can persist in protoplasm through the series of physical and chemical changes which constitute development in its dynamic aspects and how it can control and localize these changes so that an orderly whole results.

The morphological theories of polarity and symmetry which assume a stratification or localization or directed flow of so-called formative substances, or some other morphological basis, do not account for the origin of the structure assumed and meet with many difficulties, particularly in the phenomena of reconstitution of pieces.

Living protoplasms are systems in which continuous chemical and physical change takes place in a complex substratum which affects the course of this change and is affected by it. The relation between structure and function in protoplasms appears to be somewhat analogous to that existing in a stream between the banks and bed and the flowing water. If there is any truth in this conception of protoplasm, it seems entirely reasonable to believe that dynamic factors are at least as fundamental as structural factors in polarity and symmetry.

Various lines of evidence which can be mentioned only very briefly indicate that polarity and symmetry are fundamentally associated with the dynamics of protoplasm. Graded differences in rate of oxygen consumption and carbon dioxide production demonstrated by Hyman and others and of certain oxidation-reduction reactions, *e.g.*, reduction of potassium permanganate and methylene blue and the indophenol reaction, graded differences in electric potential and in susceptibility to different chemical and physical agents, and graded differences in rate of development and growth and often in structure, all agree in indicating that a physiological axis in its simplest terms is characterized by an essentially quantitative gradation in physiological condition and that rate of metabolic reactions, particularly the physiological oxida-

tions, is an essential factor in such a gradation. This evidence constitutes the basis for the terms "axial gradient," "metabolic gradient" and "physiological gradient," which explain themselves.

The question at once arises whether these quantitative gradients are actually the physiological basis of polarity and symmetry or merely indicators or results of some more fundamental condition. The experimental evidence shows first that alteration in slope or steepness of a gradient alters localization and proportions of parts in relation to that axis, second, that experimental obliteration of a gradient obliterates the corresponding polarity or symmetry from the developmental process and there is no evidence of the persistence of anything representing the axis concerned, and third, that experimental determination of a new gradient by differential exposure to light, electricity, oxygen or any other effective factor, determines a new physiological axis, provided the external factor acts long enough to determine more or less persistent protoplasmic differences at different levels.

All the evidence at present available forces us to the conclusion that polarity and symmetry of the kind which characterizes all the higher organisms, both plant and animal, and at least a large proportion of the simpler multicellular and the unicellular forms, are primarily quantitative gradients or differentials in physiological condition and that dynamic factors are at least as fundamental as any structural factors in such gradients. This conclusion does not necessarily involve the assumption that such gradients are the basis of all orderly form in living protoplasms. Stereochemical factors, colloidal phase relations, surface tension, molar pressures and tensions, and doubtless various other factors may be concerned in determining form with definite axial relations in intracellular structure, in single cells or even in some cell aggregates. But at present it seems to be true that these factors are chiefly concerned with elements of form and structure in organisms rather than with the general

axial relations of whole organisms, though it is by no means impossible that form and axial relations in some of the simpler organisms may be in large measure or wholly determined by such factors. However this may be, there is at present no actual evidence that such factors constitute the basis of the polarity and symmetry which are expressed in the orderly localization, differentiation and functional relations of axiate organisms in general. In fact, the evidence at hand indicates very clearly that such factors do not play any fundamental rôle in the establishment and maintenance of such physiological axes, though it is conceivable that they may be concerned in one way or another in some cases. In general, the physiological axis appears to be a regional quantitative differential imposed upon the protoplasmic system from without and consisting in its simplest terms of a more or less definite physiological gradient.

It may be pointed out, however, that each such gradient is a gradient in a protoplasm of specific hereditary constitution and therefore that this specific constitution is a factor in determining the modifications which the gradient may undergo during development and its final characteristics and fate, no matter how it originated or what its primary form, slope, etc. Moreover, the particular kind of organ or part which arises at a given level of an axis is determined by the specific protoplasmic constitution in reaction with the actual physiological condition existing at that level. In hydra protoplasm, for example, the hypostome and tentacles arise at the high end of the polar gradient, in frog protoplasm a tadpole head. In both cases, secondary gradients are also concerned in the formation of these and other parts, but they are different in the two forms and develop in different ways. It may also be pointed out that a gradient or gradients once established in a protoplasm may persist through cell division or other reproductive processes and so be hereditary for the resulting individual or individuals. This is the case in many processes of fission, *e.g.*, in Paramecium, in

Planaria and many other forms, and may also be the case in some animal eggs. Obviously, such inheritance involves no Lamarckian assumptions.

If physiological axes are primarily quantitative physiological gradients and if they are effective factors in determining localization and proportion of parts in individual development, alteration of the gradients in early stages must result in modifying development, and experiment shows that modification and control of development in this way are possible. Differential susceptibility to the action of physical and chemical agents in certain ranges of concentration or degree of action is a characteristic feature of physiological gradients. In the earlier stages of development and in many of the simpler organisms throughout life, that is, in all cases in which specific differentiation of different parts has not proceeded too far, this differential susceptibility is non-specific for at least a large number, if not for all external agents in certain ranges of concentration or degree of action. A comparison of the data on susceptibility with those on oxygen consumption, carbon dioxide production, reduction of permanganate, the indophenol reaction and rate of growth and development, brings to light the following relation between susceptibility and physiological condition, as characteristic of the earlier stages of development and of many of the simpler organisms throughout life. Susceptibility to concentrations or degrees of action above the range of tolerance or acclimation and either lethal in course of time or producing effects not readily reversible, varies directly with, though not necessarily proportionally to the rate of metabolic reactions, as indicated by the various methods which give data on metabolic rate. On the other hand, the ability to recover after temporary exposure to a certain range of concentration or degree of action and the ability to acclimate or acquire tolerance to a certain range of relatively low concentrations or degrees of action also varies directly with, though not necessarily proportionally to, the rate of metabolic reactions as determined or indicated by other methods.

The experimental basis for these conclusions consists of data on several hundred species of animals and plants, including among animals representatives of all the larger phyla. These data, obtained by various independent methods, agree in showing or indicating that differences in susceptibility along a physiological axis are primarily non-specific for certain ranges of concentration or degree of action of external agents and depend upon quantitative differences in physiological condition in which rate of metabolic reactions is an essential factor. In the work on susceptibility the following agents have been used: potassium cyanide; various more or less anesthetic agents including ethyl alcohol, ethyl ether, chloroform, chloretone, several of the urethanes, etc.; formaldehyde; various acids and alkalies; several alkaloids; many neutral salts, including salts of sodium, potassium, magnesium, lithium, copper, mercury and others; the vital dyes neutral red, methylene blue and Janus green; the negative condition lack of oxygen; and the physical agents, extremes of temperature, ultra-violet radiation and visible light after photochemical sensitization by means of eosin or other agents. By no means all these agents have been used on all forms examined, but in every case several from different groups have been used and it has been found that for certain ranges of concentration or degree of action, experimentally determined for each species and each agent, the susceptibility gradient representing a particular axis shows the same direction with respect to that axis for different agents, though the quantitative differences in susceptibility at different levels may differ with different agents. For the protoplasms of different species widely different concentrations or degrees of action of a particular agent are often necessary, that is to say, the susceptibility of a particular species-protoplasm to external agents in general is specific, but within the individual of the species the differences in susceptibility along an axis are primarily non-specific for different agents, at least to a very high degree.

The point must be emphasized that these differences are only primarily non-specific. As differentiation progresses, particularly in the higher animals, indications of apparent specificity of particular parts to particular agents appear and it is only among the simpler organisms that the primary non-specific differences in susceptibility persist throughout life. In highly differentiated forms such as mammals and man the susceptibilities of particular organs to particular agents appear to become specific to a high degree during the course of development.

So far as the differences in susceptibility along an axis are non-specific for different agents, the only conclusion possible is that the physiological differences on which they depend are non-specific, and if they are not specific they must be quantitative. Moreover, it has been demonstrated by other methods quite independently of work on the gradients, that quantitative physiological differences, *e.g.*, differences in rate of metabolism, do determine differences in susceptibility, and it has also been shown that quantitative differences in physiological condition are characteristic of physiological axes. In short, the evidence from susceptibility agrees with that obtained by other methods in indicating that a physiological axis is primarily a quantitative gradient involving in each case dynamic factors specific for the particular species-protoplasm and that specific regional differences along an axis arise secondarily. The evidence taken as a whole points unmistakably to a quantitative dynamic basis for polarity and symmetry and so far as I am aware, there is no actual evidence but only speculative hypotheses in support of any other conception.

By way of reply to critics, it may be noted that on the basis of the evidence non-specific differences in susceptibility along an axis can not be due merely to differences in permeability of limiting surfaces because they are similar for agents which penetrate readily through living membranes and kill by accumulation within the cells, for

agents which alter or kill the protoplasm as they penetrate and for physical agents in the action of which permeability is not concerned. Moreover, the regions most affected by high concentrations of chemical agents acclimate to or recover from the action of low concentrations most readily.

Neither can these non-specific differences in susceptibility be regarded, as some critics have assumed, as signifying that all agents act in the same way upon a given protoplasm. This is obviously not the case. Though our knowledge of the action of external agents on protoplasm is far from complete, it is sufficiently evident that different agents act in very different ways. Non-specific susceptibility apparently does not depend upon the way in which a particular agent acts, but appears to be a special case of a general relation between dynamic systems and disturbance. For present purposes this relation may be stated as follows: given a dynamic system in process of equilibration, a disturbance of any essential factor of the system adequate to produce disruption in course of time or to alter the system irreversibly will in general produce such effects more rapidly when the rate of change characteristic of the system is high than when it is low. On the other hand, when the disturbance is within the limits of tolerance or equilibration of the system, equilibration to its action, *i.e.*, acclimation, or recovery from its effects will occur more rapidly in the system with high than in that with low rate of change. These relations hold because in both cases the rate of change characteristic of the system becomes a factor in determining the effects of the disturbance upon the whole. For example, a barrier thrown across a flowing stream, a diversion channel or a filling of the bed will produce its effects, *e.g.*, formation of a lake, diversion, draining of the bed below, etc., more rapidly in the rapid than in the slow stream, and equilibration to the presence of a slight obstacle or recovery from the effects of a temporary disturbance will also occur more rapidly in the rapid than in the slow stream.

The existence of quantitative gradients in physiological condition along the axes of organisms and the nonspecific differential susceptibilities of different levels of these gradients provide a physiological basis for the modification and control of morphogenesis by means of external physical and chemical agents and for the interpretation of the modifications produced. On this basis the following modifications are possible: First, with relatively high concentrations or degrees of action of inhibiting agents which must be experimentally determined for each species-protoplasm and each agent, differential inhibition is produced, the degree of inhibition of development varying with the activity and susceptibility at different levels of the axis or axes; second, with accelerating agents it is possible within certain limits to produce differential acceleration, the degree of acceleration and enlargement varying with the susceptibility at different levels; third, with continuous exposure to a certain range of low concentration or degree of action, differential acclimation is possible, the rate and degree of acclimation and therefore of change in rate of metabolism and development varying with the activity at different levels; fourth, differential recovery follows temporary exposure to certain concentrations or degrees of action, the rate and degree of recovery varying with the activity at different levels. In differential inhibition the regions of greatest activity, apical or anterior and median ventral in most bilateral invertebrates, median dorsal in vertebrates, are most inhibited and therefore least developed, but with certain degrees of differential inhibition the less active and therefore less inhibited regions show hyperplasia because, under the altered metabolic relations, they are able to obtain a larger proportion of the nutritive material available. In certain degrees of differential acceleration, acclimation and recovery the changes in form and proportion are in the opposite direction.

These modifications are not specific for different agents, and even in different species and groups they

show the same differentials with respect to the axis or axes present. In this connection it is of great interest to note that in the history of developmental physiology there are no well-established cases of developmental modification in animals resulting from uniform exposure of the whole embryo at early stages to action of an external agent, which are specific for a particular agent. Cases originally regarded as specific effects of particular agents have been shown on further work to be non-specific. For example, the exogastrula, at first supposed by Herbst to be a specific effect of lithium, can be produced by many different agents of very different constitution and method of action and is now known to be primarily a result of certain degrees of differential inhibition. Again, cyclopia in fishes, the "magnesium embryo" of Stockard, is now known not to be specific for magnesium, but to result from the action of many different agents and is likewise a result of differential inhibition. Moreover, various investigators in the past have expressed the belief that the action of external agents on development is at least not commonly specific. More than 30 years ago Dareste pointed out that the same sorts of developmental modifications were produced in the chick by various external agents. The work under discussion here is then directly in line with earlier work, but attempts to go one step further in providing on the basis of many different lines of evidence a general physiological interpretation of the observed facts.

Modification of embryonic development through differential susceptibility to external agents in relation to the physiological gradients has been studied in hydroids (Child), sea urchin, starfish and sand dollar (Child, MacArthur, Hinrichs), annelids and ascidians (Child), fishes (Gowanloch) and amphibia (Bellamy), and the data of earlier investigators are essentially contributions to the same subject. The production of non-specific modifications in reconstitutional development in Planaria through differential susceptibility to external agents in relation to

the physiological gradients has been a feature of laboratory class work for a number of years.

Because of the large amount of work done on modification of larval development in the sea urchins, this form serves best as a basis for concrete illustration of the directions, relation to the gradients and non-specific character for different agents of the modifications, but brief comparative reference is made to developmental modifications in the hydroid, the frog and the reconstitutional development of pieces of Planaria.[2]

In the early sea urchin blastula the gradient, as determined by susceptibility to many chemical agents, to lack of oxygen, to light, etc., and by rate of oxidation-reduction reactions, is apico-basal, the high end being apical, but as development progresses the high activity of the apical region extends along a meridian which becomes median anterior in the later larva. Consequently, in the later gastrula, the prepluteus and early pluteus activity and susceptibility decrease in all directions from apical and median anterior regions.

Exposure of the early stages to concentrations of external agents which inhibit development results first in a greater inhibition of apical as compared with basal region and later of apical and median anterior as compared with basal, lateral and posterior. All degrees of differential inhibition can be produced, ranging from forms with slightly reduced apical region (oral lobe) and small angle between the arms, through forms with parallel arms, with arms fused in the median line, to the complete obliteration of bilaterality and finally of polarity also.

Exposure to a certain range of lower concentrations or degrees of action or temporary exposure followed by return to sea water produces at first some degree of differential inhibition, but later a more rapid acclimation or recovery of the more active apical and median anterior regions occurs and with certain degrees of such differential

[2] In the original presentation of the paper the developmental modifications discussed were illustrated by charts.

recovery the proportions of the larva are altered in directions opposite to those characteristic of differential inhibition. Differential accelerations produced by Dr. Hinrichs with certain concentrations of caffein resemble in form the differential acclimations and recoveries, but are usually of large size, often larger than normal, because there is no primary inhibition. In the work on the sea urchin the following agents have been used: potassium cyanide, ethyl alcohol, ether and various other anesthetics; acids, alkalies, the alkaloid caffein, many neutral salts, including salts of mercury, copper, lithium, magnesium, sodium, etc.; extremes of temperature, ultra-violet radiation and visible light after sensitization by eosin.

The various types of modification described above do not represent individual cases selected from the experimental cultures: they are the only types of modification found. Individual differences in susceptibility of different eggs determine a certain range of modification in any culture, but after the proper ranges of concentration or degree of action are determined it is easily possible to control the modifications so that all individuals of a culture show, for example, some degree of differential inhibition or differential acclimation or differential recovery. All modifications produced thus far by all agents used fall into one of the four groups, but they differ in degree with different agents according to the rapidity with which particular agents produce their effects, the amount of difference in susceptibility at different levels of a gradient and the rapidity of acclimation and recovery. For example, exogastrulation represents a differential inhibition, but this modification is most readily produced by agents to which the differences in susceptibility at different levels of the apico-basal gradient are large. Lithium salts, so far as tested, are such agents, but some degree of exogastrulation can be produced by many, perhaps by all external agents with proper concentration and treatment.

In the hydroid only an apico-basal gradient is present

in early stages, but later a second gradient, opposite in direction to the first, arises at the basal end of the planula. This new gradient represents a process of budding and gives rise to the first hydranth. As in the sea urchin, exposure to inhibiting agents in the earlier stages of hydroid development results in a greater degree of inhibition of apical than of basal regions and the latter show hyperplasia when the inhibiting action is not too great. This basal hyperplasia often gives rise to forms somewhat similar to exogastrulae. More extreme degrees of differential inhibition obliterate polarity completely, and the larva remains or becomes spherical and never develops any indications of an axis, though it may live for a long time.

As regards later stages, certain degrees of differential inhibition transform the hydranth-stem axis into a creeping stolon, and certain degrees of differential acclimation and recovery transform stolons into hydranth and stem. In differential acclimation or recovery of the planula, if polarity has not been completely obliterated, outgrowth may occur at one or both ends of the planula, according as one or both gradients are present. Such outgrowth is usually at first a stolon, but later may transform into hydranth and stem if the degree of acclimation or recovery is sufficient. When polarity is completely obliterated through differential inhibition, return to sea water is usually followed by the determination of one or more new gradients and axes in consequence of the differences in condition of cells on the free surface and those on the surface in contact as the cell mass lies on the bottom or in consequence of slight local differences in different regions of the mass ("adventitious" polarities). All these modifications have been produced by potassium cyanide, increase in hydrogen ion concentration, lithium chloride and neutral red and the same modifications have been observed in several species. In some species development in standing water or in water with a slightly increased carbon dioxide content or insufficient aeration produces

differential inhibition. No other modifications than those mentioned have been observed in any case.

In the frog an apico-basal gradient is characteristic of the earlier stages, as Bellamy has shown, but as gastrulation approaches the dorsal lip region becomes highly active and after closure of the blastopore the regions of highest activity and susceptibility are anterior and median dorsal, with a secondary gradient arising posteriorly as the tail bud appears. The action of inhibiting agents on early cleavage stages results in greater inhibition of apical than of basal regions and accelerating agents accelerate apical more than basal regions. In later stages, after the dorsal lip region has become active, inhibiting agents inhibit this region more than others, the form of the larva depending on the stage and period of exposure and the concentration or degree of action of the agent. Permanent yolk plugs, spina bifida, equatorial gastrulation and obliteration of bilateral symmetry all represent various degrees of differential inhibition. Still later stages show all degrees of microcephaly to almost complete acephaly, the tail is more or less inhibited and grows dorso-posteriorly instead of posteriorly because of inhibition of the dorsal region. In relation to bilaterality differential inhibition results, according to its degree in approximation to the median line of lateral organs, *e.g.*, ventral suckers, nasal primordia, eyes or in formation of a single organ in the median line, or in still more extreme cases in complete absence of these organs.

In differential acclimation and differential recovery some degree of differential inhibition in the earlier stages is followed by the more rapid acclimation or recovery of the more active regions, so that the later stages show modification opposite in direction to the results of differential inhibition. Differential acceleration produces megacephalic forms with eyes and other lateral organs far apart and with long tails.

In the work on the frog the following agents were used: potassium cyanide, formaldehyde, potassium per-

manganate, mercuric chloride, lithium chloride, hydrochloric acid, sodium hydroxide and ethyl alcohol, and the types of modification are similar to those obtained by various other investigators with some of these and with other agents. As regards relations to the axial gradients, the modifications are not specific for the different agents, but their character depends on concentration of agent, period and stage of exposure and difference in susceptibility to different agents of different levels of the gradients.

Mr. Gowanloch has obtained essentially similar series of modifications in fishes with a large number of agents, but his work is not yet published. In passing, attention may be called again to the fact that cyclopia, which Stockard formerly regarded as a specific effect of magnesium on the fish embryo, is simply a result of differential susceptibility between median and lateral regions of the head and can be produced in fishes, amphibia and many other forms by many different agents. Stockard's later interpretation of cyclopia and related modifications is along the same lines as my own, except that he seems to regard rate of development as something fundamental in itself. The evidence indicates, however, that rate of development is dependent on rate of metabolism or of certain metabolic reactions.

In the reconstitution of pieces of Planaria various types of anterior end, normal, teratophthalmic, teratomorphic, anophthalmic and acephalic appear. Under the usual conditions the frequency of the various types depends on physiological conditions in the piece, but all the types can be produced by all chemical and physical agents used thus far and various lines of evidence show that they represent different degrees of differential inhibition, resulting from the differential susceptibility of median and lateral regions. All degrees of approximation of the eyes, even to cyclopia and to anophthalmia, occur according to the degree of differential inhibition. On the other hand, under conditions which permit differ-

ential acclimation or differential recovery the median region of the head may show considerable overgrowth and heads which at first possess only a single median eye may later develop a right and left eye in addition. These modifications of the head in Planaria have been produced with potassium cyanide, various anesthetics, acids, alkalies, caffein and extremes of temperature. They show no indication of specificity for particular agents, and their relation to the gradients is the same as in other forms. Cyclopia in Planaria, for example, is no more specific for any particular agent than in fishes or in amphibia, and it is the same sort of modification physiologically as the fusion in the median line of the two anal arms of the sea urchin larva.

Many other data might be presented, but these are perhaps sufficient to indicate the importance of differential susceptibility in modification of development in widely different forms and the relations between differential susceptibility and the axial gradients. The evidence forces us to the conclusion that the primary physiological differences at different levels of an axis are quantitative differences in which the metabolism characteristic of the species is a fundamental factor and that the specific differentiations of different regions and the proportions of the individual are secondary results of these differences.

ON THE RELATIONSHIP BETWEEN STATURE AND THE LENGTH OF THE APPENDAGES IN MAN

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THE change in the relative length of the trunk and the appendages during the development of the individual is a phenomenon quite familiar to anthropologists. While numerical illustrations and graphical representations of the changes in the relative proportions of the head, trunk and lower limbs with growth are to be found in many works on anthropology, the problem of the proportionality of the various parts of the body does not seem to have received the amount, or at least the kind, of attention which it deserves. The investigation of the problem can and should be carried much further. It seems important in its relationship to the physiology of development to determine whether *at any given age* the physical proportions of the individuals of a population are such as might have arisen from a retardation or cessation of growth at varying antecedent stages of development. In short, the analysis must not be limited to the comparison of average dimensions of individuals of different ages, but should be extended to groups of individuals of the same age.

The interest of the problem is not limited to its relation to morphogenesis merely. As shown by the classical studies of Galton and Pearson, stature and other dimensional characters are inherited. Castle (1914) has taken up experimentally and statistically the problem of the extent to which the inheritance of the size of the different parts of the individual is due to general growth factors. Davenport (1917) concludes that there is evidence that the elements of stature are to a certain extent separately inheritable, and further that the inheritance of the pro-

portional lengths of the elements of stature is as evident as the inheritance of the absolute dimensions. In its relation to the problem of inheritance it is therefore desirable to determine whether the proportionality of the parts at any given stage of development is dependent upon their absolute size.

Failure to have considered these problems is perhaps largely due to the fact that extensive series of data and refined quantitative methods of analysis are essential prerequisites. As far as I am aware the most extensive data on the relationship between the total height of the individuals and the amount contributed to this total by one of the elements of stature in a relatively homogeneous series of individuals are the series of correlations between total stature and sitting height in school children of various ages published by Boas and Wissler (1906) and the more recent determination of the correlations between stature and total leg and thigh length in Oxford students due to Schuster (1911).

But stature as a whole is made up of head and trunk length and leg length. On *a priori* grounds one would, therefore, have expected the correlations found by Boas and Wissler and by Schuster to be high. Those published by Boas and Wissler for stature and sitting height are of the order .7–.9. Those measuring the closeness of correlation between stature and leg and thigh length in Oxford students are of the order .8 for the thigh and .9 for the whole leg.

Since common observation and the diagrams showing the change in the proportionality of the elements of stature in text-books on anthropology show that in the later stages of development the greatest source of increase in stature is the growth of the lower limbs, it is difficult to see why anthropologists have taken sitting height (used as a measure of trunk + head length) rather than leg length, which may *for purposes of rough approximation* be taken as the difference between total height and sitting height, in determining their correlations.

While the published data are given in the form to determine the correlation between total stature and sitting height, it is quite possible to determine the correlations for total stature and leg length (Harris, 1917). Since our primary purpose is not a discussion of the correlation between absolute dimensions these coefficients will not be discussed here.

While the coefficients showing the relationship between stature and sitting height or stature and leg length as deduced by Boas and Wissler, Schuster and others are of great value in that they express a relationship which had heretofore been merely a vague conception on a universally comparable quantitative scale, they fail to give all (and, indeed, the most important) information concerning the interrelationship of these variables.

The first requisite for progress in the analytical as compared with the purely descriptive treatment of such problems seems to be a formula which will measure the relationship between total stature and the proportional length of any of its constituents.

It is obvious that tall children or adults have on the average longer legs than short ones. Do they have relatively longer legs in populations at each stage of development?

Schuster (1911), in determining the correlation between stature and the ratio $\frac{\text{length of leg}}{\text{stature}}$ has recognized the need of such analysis. His results will be noted below.

I believe the correct method for dealing with all such problems to be that worked out several years ago by Professor Pearson and myself and since then rather widely applied to biological problems (Harris 1909, 1918).

This coefficient measures the relationship between a variable, such as stature, and the deviation of a dependent variable, such as sitting height, from its probable value on the assumption that the two variables should bear the same proportional relationship to each other

throughout their ranges of variation, *i.e.*, that in any individual case, $s = ph$ where $p = \bar{s}/\bar{h}$, the bars denoting the means of the independent variable h = height, and of the dependent variable s, = sitting height; s' is the theoretical value of s, and the correlation between h and z, $= s - s'$, or the deviation of sitting height from its probable value, shows whether s becomes relatively larger or smaller with variations of h above or below the population mean.

A convenient working formula is

$$r_{hz} = \frac{r_{hs} - V_h/V_s}{\sqrt{1 - r_{hs}^2 + (r_{hs} - V_h/V_s)^2}}$$

where $V_h = \sigma_h/\bar{h}_h$, $V_s = \sigma_s/\bar{s}$, the sigmas denoting standard deviations and the bars the means of the two characters.

In working with the data of Boas and Wissler we have the advantage of dealing with the individuals in a number of different age groups, characterized by the increasing dimensions of each of the characters. The great regularity of the increase in the average dimensions of the subjects is shown in diagram 1 which represents the change in the measurements of Worcester boys with increasing age. The straight lines are fitted to the means. Probably the irregularity in the rate of increase in total stature noticeable at 14 to 16 years is due to the onset of puberty.

The curve for growth in stature found by Bowditch for Boston school children and reproduced by Minot (1908) indicates essential linearity until the age of 12 to 14 years when the onset of puberty introduced disturbing factors. The same is true of the growth of Berlin school children as illustrated by Martin.

From Table I*a* and I*b* of Boas and Wissler we abstract the means and standard deviations of stature and sitting height in Worcester boys and girls for each year of age from 6 to 17. The correlations between stature and sitting height deduced by Boas and Wissler are given in their Tables II*a* and II*b*. Inserting these values in our

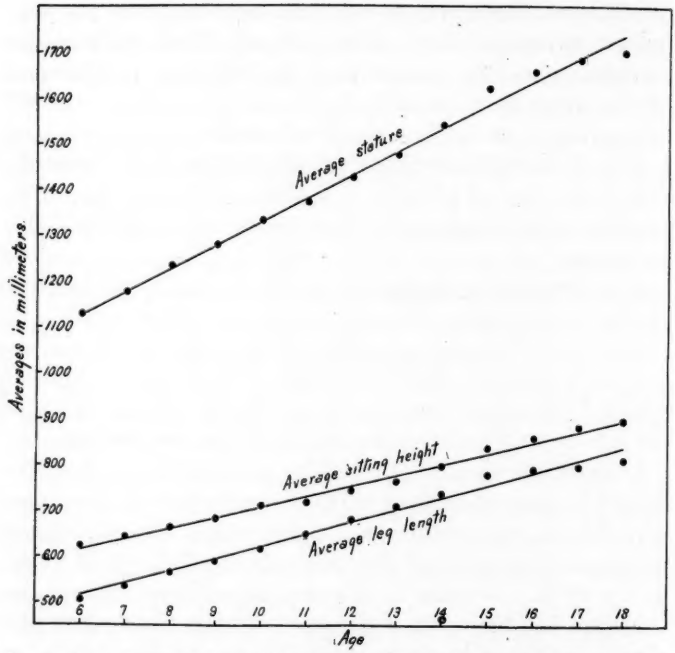

DIAGRAM 1. Regression of sitting height, leg length and stature on age in Worcester boys.

formula, we obtain the correlations between stature and the deviation of sitting height from its probable value. These are laid beside the correlations between stature and sitting height published by Boas and Wissler in table I. Without exception the coefficients are negative in sign. They range from —.13 to —.47, with a general average of —.256 for boys, and from —.10 to —.48 with a general average of —.292 for girls.[1]

The constants for more extensive data for Milwaukee and Toronto boys and girls may be deduced from Tables VI*a*, VI*b* and VII*a* of Boas and Wissler. The results in Table II fully confirm the conclusions drawn from Table I.

[1] These averages are obtained without weighting. If the contestants be weighted with the number of children upon which they are based the results are essentially the same, *i.e.*, —.258 for boys and —.276 for girls.

Unfortunately, Boas and Wissler have not recorded their statistical constants to enough places to give smooth results when they are used for further calculations.

TABLE I

RELATIONSHIP BETWEEN STATURE AND SITTING HEIGHT IN WORCESTER BOYS AND GIRLS

Age	Boys				Girls			
	N	Correlation between stature and sitting height	Correlation between stature and deviation of sitting height	$\frac{r}{E_r}$	N	Correlation between stature and sitting height	Correlation between stature and deviation of sitting height	$\frac{r}{E_r}$
6	110	.78 ± .03	—.47 ± .05	9.4	99	.67 ± .04	—.46 ± .05	9.2
7	203	.81 ± .02	—.22 ± .05	4.4	129	.74 ± .03	—.42 ± .05	8.4
8	187	.83 ± .02	—.37 ± .04	9.3	146	.81 ± .02	—.32 ± .05	6.4
9	225	.79 ± .02	—.29 ± .04	7.3	152	.81 ± .02	—.35 ± .05	7.0
10	228	.80 ± .02	—.33 ± .04	8.3	174	.84 ± .02	—.30 ± .05	6.0
11	211	.88 ± .01	—.13 ± .05	2.6	202	.83 ± .02	—.19 ± .05	3.8
12	256	.88 ± .01	—.15 ± .04	3.8	208	.84 ± .01	—.31 ± .04	7.8
13	224	.90 ± .01	—.27 ± .04	6.8	190	.80 ± .02	—.10 ± .05	2.0
14	228	.92 ± .01	—.30 ± .04	7.5	151	.85 ± .02	—.19 ± .05	3.8
15	183	.85 ± .01	—.17 ± .05	3.4	138	.81 ± .02	—.12 ± .06	2.0
16	116	.79 ± .02	—.27 ± .06	4.5	101	.71 ± .03	—.48 ± .05	9.6
17	51	.77 ± .04	—.18 ± .09	2.0	69	.71 ± .04	—.26 ± .08	3.3

TABLE II

RELATIONSHIP BETWEEN STATURE AND SITTING HEIGHT IN MILWAUKEE AND TORONTO BOYS AND GIRLS

Age*	Boys				Girls			
	N	Correlation between stature and sitting height	Correlation between stature and deviation of sitting height	$\frac{r}{E_r}$	N	Correlation between stature and sitting height	Correlation between stature and deviation of sitting height	$\frac{r}{E_r}$
M. 5	296	.73 ± .02	—.32 ± .04	8.0	250	.77 ± .02	—.34 ± .04	8.5
T. 7	910	.76 ± .01	—.23 ± .02	11.5	800	.73 ± .01	—.25 ± .02	12.5
M. 7	641	.84 ± .01	—.33 ± .02	16.5	586	.85 ± .01	—.25 ± .03	8.3
T. 10	853	.78 ± .01	—.36 ± .02	18.0	848	.79 ± .01	—.22 ± .02	11.0
M. 10	553	.80 ± .01	—.42 ± .02	21.0	553	.93 ± .00	—.16 ± .03	5.3
T. 13	600	.80 ± .01	—.41 ± .02	20.5	675	.72 ± .01	—.10 ± .03	3.3
M. 13	365	.95 ± .00	—.16 ± .03	5.3	333	.85 ± .01	—.35 ± .03	11.7
M. 16	58	.83 ± .03	—.05 ± .09	0.6	108	.62 ± .04	—.09 ± .06	1.5

* M = Milwaukee, T = Toronto.

Furthermore, the number of individuals upon which their constants for total stature and sitting height are based sometimes differs. This introduces another possible source of error. While the consistency of the end results in Tables I and II is such as to make it practically certain that these sources of error are of little practical importance, I have thought it worth while to draw from their tables of actual measurements of boys (pages 49–132) a series of individuals in which the record of stature, sitting height and weight are all included.[2] The results of calculations based on these original measurements are given in Table III.

TABLE III

RELATIONSHIP BETWEEN STATURE AND SITTING HEIGHT IN WORCESTER BOYS

Age	N	Total height and sitting height	$\frac{r}{E_r}$	Total height and deviation of sitting height from its probable value	$\frac{r}{E_r}$
6	104	.86 ± .02	48.3	—.25 ± .06	4.1
7	144	.74 ± .03	29.0	—.27 ± .05	5.2
8	111	.85 ± .02	47.1	—.24 ± .06	4.0
9	133	.79 ± .02	36.1	—.27 ± .05	4.9
10	122	.82 ± .02	41.8	—.32 ± .05	5.8
11	113	.80 ± .02	35.1	—.39 ± .05	7.4
12	147	.89 ± .01	76.0	—.22 ± .05	4.1
13	129	.86 ± .02	56.3	—.38 ± .05	7.4
14	128	.91 ± .01	85.6	—.19 ± .06	3.3
15	140	.92 ± .01	105.7	—.10 ± .06	1.8
16	92	.78 ± .03	28.1	—.29 ± .06	4.5
17	47	.78 ± .04	20.0	—.31 ± .09	3.5
18	48	.85 ± .03	30.9	—.16 ± .09	1.7

The values are in excellent general agreement with those based on the constants of Boas and Wissler.

To test the influence of age heterogeneity at this period of relatively rapid development upon the relationship under investigation we have grouped the series of data upon which the results in Table III have been based into two-year classes. The coefficients are given in Table IV.

The results show that the correlation between stature and sitting height for two-year groups lies between that

[2] The records in the table of repeated measurements were not included. Unfortunately, there are evidences of typographical errors in these protocols which cast some doubt upon the exactness of constants based upon them.

for the single year periods in the earlier years, but is larger than that of either of the two years in the constants determined for 14–17 years. Whether this result is generally valid or whether it is a peculiarity of these series of data can not be asserted without further work.

In the correlation between stature and the deviation of sitting height from its probable value the coefficients in the earlier pairs of years are generally larger than either of the two constituent years. In the later pairs of years they are intermediate.

TABLE IV

RELATIONSHIP BETWEEN STATURE AND SITTING HEIGHT IN WORCESTER BOYS CLASSIFIED IN TWO-YEAR AGE GROUPS

Age	N	Total height and sitting height	$\frac{r}{E_r}$	Total height and deviation of sitting height from its probable value	$\frac{r}{E_r}$
6 and 7	248	.82 ± .01	82.0	—.28 ± .04	7.0
8 and 9	244	.83 ± .01	83.0	—.29 ± .04	7.3
10 and 11	235	.80 ± .02	40.0	—.42 ± .04	10.5
12 and 13	276	.88 ± .01	88.0	—.33 ± .04	8.3
14 and 15	268	.93 ± .01	93.0	—.14 ± .04	3.5
16 and 17	187	.82 ± .02	41.0	—.20 ± .05	4.0

Since the values of the correlations between stature and the deviation of sitting height from its probable value are not very greatly different in groups of one year and of two year age range, it is evident that this relationship is largely due to differentiation occurring earlier in life than the age groups covered by these data, and not primarily to age heterogeneity within the groups under investigation.

As a further test of the validity of the conclusions here drawn I have determined the relationships on small groups of individuals selected from the series of Boas and Wissler because measurements of forearm, to be discussed later, were also available.[3]

The essential constants set forth in Table V are fully confirmatory of those already given.

[3] These are children given in their repeated measurement tables.

Schuster's correlations are based on stature and leg length, not on stature and sitting height. His constants and those which have been deduced from them are laid side by side in Table VI.

TABLE V

RELATIONSHIP BETWEEN STATURE AND SITTING HEIGHT IN WORCESTER BOYS

Age	Number	Correlation between stature and sitting height	Correlation between stature and deviation of sitting height from its probable value
7	42	.86 ± .03	−.43 ± .09
8	63	.80 ± .03	−.32 ± .08
9	67	.73 ± .04	−.08 ± .08
10	81	.81 ± .03	−.30 ± .07
11	72	.79 ± .03	−.15 ± .08
12	71	.87 ± .02	−.38 ± .07
13	76	.87 ± .02	−.07 ± .08
14	60	.84 ± .03	−.32 ± .08

TABLE VI

RELATIONSHIP BETWEEN STATURE AND LENGTH OF LOWER APPENDAGES IN OXFORD STUDENTS

Age	Stature and leg r_{hl}	Stature and thigh r_{ht}	$r_{hl}-r_{ht}$	Stature and deviation of leg r_{hz_l}	Stature and deviation of thigh r_{hz_t}	$r_{hz_l}-r_{hz_t}$
18	.87 ± .01	.80 ± .02	+.07 ± .02	.42 ± .05	.47 ± .04	−.05 ± .06
19	.86 ± .01	.74 ± .02	+.12 ± .02	.35 ± .03	.26 ± .03	+.09 ± .04
20	.89 ± .01	.82 ± .02	+.07 ± .02	.39 ± .04	.36 ± .04	+.03 ± .06
21	.91 ± .01	.82 ± .02	+.11 ± .02	.48 ± .04	.34 ± .05	+.14 ± .06
22	.91 ± .01	.83 ± .02	+.11 ± .02	.40 ± .06	.34 ± .06	+.06 ± .09

Note that for both stature and length of leg and stature and length of thigh the correlations are high. Those for stature and length of leg are uniformly higher than those for stature and length of thigh, just as one might expect them to be on morphological grounds.

Since the correlations for stature and the deviation of sitting height from its probable value is negative, one would expect the correlation between stature and the deviation of leg length from its probable value to be posi-

tive. This is found to be the case. These coefficients are about half as large as those given by Schuster for the relationship between the actual values of the same variables. Thus taller individuals have relatively as well as absolutely longer legs and thighs than short ones.

It is of interest to compare the correlation between stature and the deviation of leg length, z_l, and thigh length, z_t. Except for young men of 18 years the correlation between stature and the deviation of total leg length from its probable value is higher than that between stature and the deviation of thigh length from its probable value.

As noted above, Schuster has investigated the same relationship for total leg length by determining the correlation between stature and the ratio $\frac{\text{length of leg}}{\text{stature}}$. His results may be placed beside our own.

	Stature and ratio	Stature and deviation
Age 18	$.44 \pm .05$	$.42 \pm .05$
Age 19	$.37 \pm .03$	$.35 \pm .03$
Age 20	$.42 \pm .04$	$.39 \pm .04$
Age 21	$.50 \pm .04$	$.48 \pm .04$
Age 22	$.45 \pm .06$	$.40 \pm .06$

Uniformly $r_{hr} > r_{hz_l}$. A discussion of the whole problem would lead us quite too far into the question of correlation of indices.

The relationship between stature and sitting height will be more clearly brought out by representing the increase in the absolute sitting height and the decrease in the relative sitting height with increase in stature by straight line equations.

Utilizing the series combined in two year age groups in order to have larger numbers of individuals upon which to base averages, we have the equations and the empirical mean sitting heights represented graphically in diagram 2. This shows that the increase in sitting height with increase in stature may be excellently represented by the slope of a straight line.

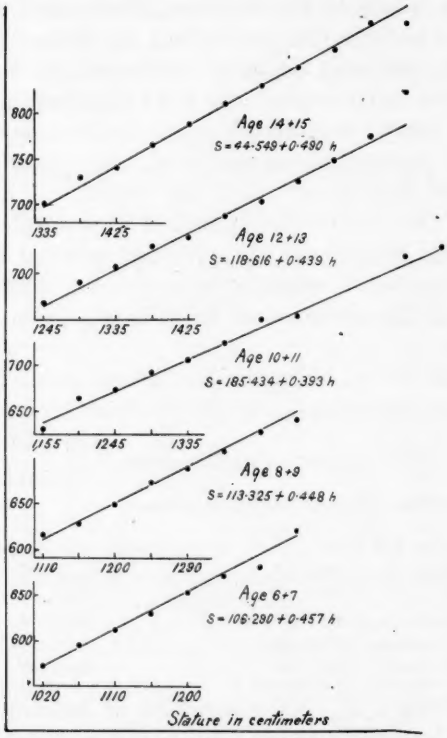

DIAGRAM 2. Regression of sitting height (ordinates) on stature in Worcester boys.

The equations showing the relationship between stature and the deviation of sitting height from its probable value and between stature and the deviation of leg length ($=h-s$, approximately) from its probable value are interchangeable with changes of sign only. Those for the regression of the deviation of sitting height from its probable value on stature are:

Ages 6 and 7	$z = 108.890 - 0.094\,h$
Ages 8 and 9	$z = 106.068 - 0.084\,h$
Ages 10 and 11	$z = 186.971 - 0.138\,h$
Ages 12 and 13	$z = 113.466 - 0.078\,h$
Ages 14 and 15	$z = 43.301 - 0.027\,h$

The equations for the regression of the deviation of leg length from its probable value on stature are given and are represented graphically on Diagram 3.

These lines, showing the theoretical mean deviation of the leg lengths of individuals of different statures from

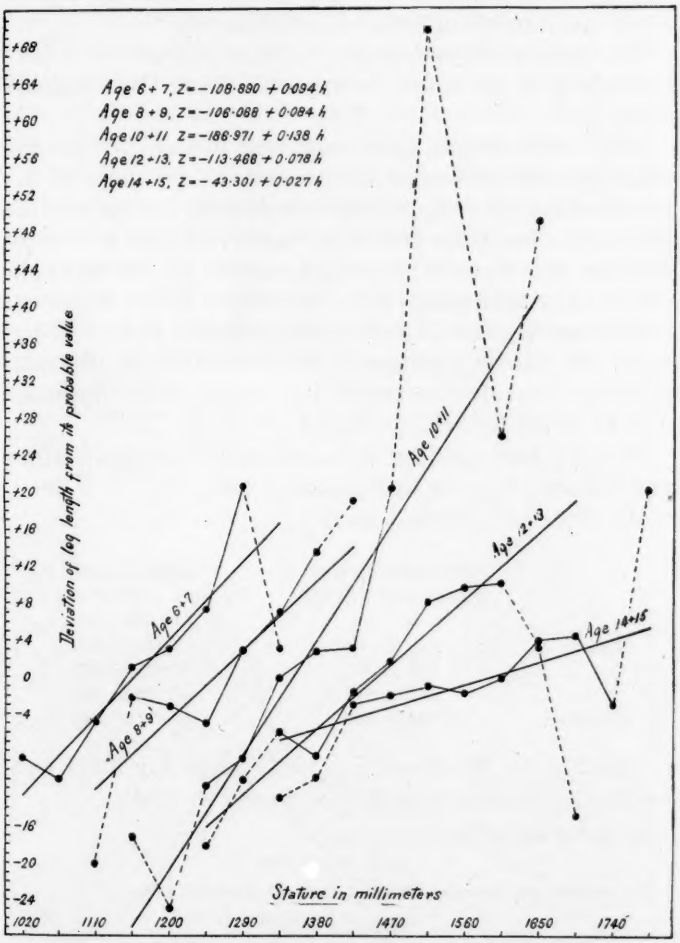

DIAGRAM 3. Regression of the deviation of leg length from its probable value on stature in Worcester boys.

their probable value, show a decrease in the relative length of sitting height and an increase in the relative leg length with increase in stature in groups of individuals of two year age range.

The empirical means are very widely scattered about the lines, especially in the case of the stature groups in which the number of observations is small.

The general agreement of the lines and means is perhaps as good as might be expected from the available data.

While the formula here employed to measure the relationship between total stature and the deviation of the length of the lower appendages from their probable value has heretofore been limited to cases in which y is some fraction of x it seems quite legitimate to extend its application to at least some cases in which y is not an actual constituent part of x but considered merely in its relation to x. If this be legitimate, we may consider the relationship between stature and the length of the upper as well as of the lower appendages.

We may first consider the correlations between stature and forearm, c, given by Pearson (1903).

We find the following values:

	Correlation between Stature and Forearm r_{hc}	Correlation, Stature and Deviation of Forearm from its probable value, r_{hz_c}
Father	.642 ± .012	—.149 ± .020
Son	.686 ± .011	—.086 ± .020
Mother	.597 ± .013	—.171 ± .020
Daughter	.704 ± .009	—.071 ± .018

Turning to Macdonell's (1902) data for 3000 non-habitual criminals from Scotland yard we find:

For stature and cubit, c.

$$r_{hc} = .800 \pm .004$$

For stature and deviation of cubit from its probable value

$$r_{hz_c} = -.149 \pm .012$$

Turning to Orensteen's (1915) measurements of Cairo-born natives, I have deduced from his Table XIV,

For stature and left cubit,

$$r_{hc} = .772 \pm .010$$

For stature and deviation of left cubit from its probable length,

$$r_{hz_c} = .002 \pm .024$$

Summarizing these results for stature and length of cubit in adults, we note that while all the correlations between stature and length of forearm are of the order $r = .60$ to $r = .80$, the correlations between stature and the deviation of the length of cubit from its probable value are low. Five of the six coefficients are negative in sign. These five are from about four to nearly ten times as large as their probable errors and may be reasonably regarded as statistically significant. The single positive constant is smaller than its probable error.

Expressing the change in the relative length of the forearm in terms of regression, we have the results for two of Pearson's series represented with the actual mean deviations of forearm length from its probable value in diagram 4.

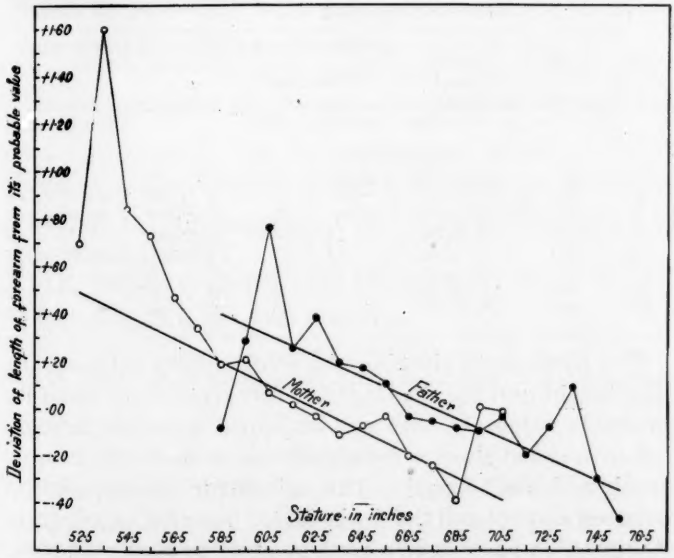

DIAGRAM 4. Regression of deviation of length of forearm from its probable value on stature in Pearson's series of adults.

Turning to the small groups of children for which Boas and Wissler have given data for length of forearm, we have the computed values for the interrelationship of sitting height and length of forearm and for stature and length of forearm given in Table VII.

The great irregularity of the results is perhaps largely due to the small numbers of children available and the untrustworthiness of some of the published measurements, as already indicated.

It is to be noted first of all that the correlations between stature and forearm are higher than those between sitting height and forearm. This means merely that prediction of forearm can be made more exactly from the length of the trunk and the length of the leg than from one of these dimensions alone.

TABLE VII

RELATIONSHIP BETWEEN STATURE AND SITTING HEIGHT AND THE LENGTH OF THE APPENDAGES IN WORCESTER BOYS

Age	Number	Correlation for sitting height		Correlation for stature	
		Sitting height and forearm	Sitting height and deviation of forearm from its probable value	Stature and forearm	Stature and deviation of forearm from its probable value
7	42	.69 ± .05	+ .21 ± .10	.77 ± .04	+ .27 ± .10
8	63	.53 ± .06	− .10 ± .08	.58 ± .06	− .03 ± .08
9	67	.63 ± .05	− .65 ± .05	.83 ± .03	− .31 ± .07
10	81	.54 ± .05	− .11 ± .07	.71 ± .04	+ .12 ± .07
11	72	.50 ± .06	− .34 ± .07	.72 ± .04	+ .00 ± .08
12	71	.76 ± .03	− .15 ± .08	.87 ± .02	− .11 ± .08
13	76	.71 ± .04	− .10 ± .08	.84 ± .02	+ .24 ± .07
14	60	.69 ± .04	− .15 ± .09	.87 ± .02	+ .11 ± .09

The coefficients showing the relationship between sitting height and the deviation of forearm length from its probable value are with one exception negative in sign, indicating that shorter forearm is associated with greater trunk and head length. The values for the correlation between stature and the deviation of forearm length from its probable value are in part positive in sign. They do not justify any conclusion concerning the variations in

the relative length of the forearm with variation in stature other than that the relationship, if it exists at all, is very small.

Table V, which gives the relationship between stature and the deviation of sitting height from its probable value in the same series of individuals, shows that the latter relationships are clearly significant. It is evident, therefore, that the relationships between stature and the upper and lower appendages are of a very different nature.

Summarizing the results of all the data for length of forearm we may conclude that if there be any relationship at all between stature and the relative length of the forearm it is of a negative kind, relatively shorter forearm being associated with greater stature. Thus the dimensions of the upper and lower appendages show a quite different relationship to total stature.

Macdonell (1902) has also given data for the relationship between stature and the length of the left middle finger. The results are:

For stature and length of left middle finger, f.

$$r_{hf} = 0.661 \pm 0.007$$

For stature and deviation of length of left middle finger from its probable value,

$$r_{hz_f} = -0.204 \pm 0.012$$

We may also utilize the 802 measurements of stature and left middle finger in Cairo-born natives given by Orensteen (1915).

The results deduced from his Table XI are:

For stature and length of left middle finger,

$$r_{hf} = 0.637 \pm 0.014$$

For stature and deviation of left middle finger from its probable length,

$$r_{hz_f} = -0.037 \pm 0.024$$

Summary

The purpose of this paper has been to consider the relationship between stature and the length of the upper and lower appendages in man. The data represent both children and adults of both sexes.

The correlation between stature and the length of the appendages is fairly high. In the case of the lower appendages this relationship is the inevitable result of the fact that the appendages themselves are one of the chief components of stature.

While the coefficient of correlation measuring this relationship has descriptive value, it fails to bring out fully the morphological relationship between these variables. A coefficient showing the relationship between stature and the deviation of the components (sitting height, leg length or thigh length) from their probable value shows that in tall individuals sitting height forms a relatively smaller and leg length a relatively larger proportion of total stature than in short ones. This relationship holds for both adults and children of various ages.

The relationship between stature and the deviation of the length of the upper appendages from their probable value is relatively small and in some series of data can not be considered significant in comparison with its probable error. The coefficients for the anterior appendages are prevailingly negative in sign.

Thus anterior and posterior appendages show quite different relations to total stature.

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ABERRANT ENDOSPERM DEVELOPMENT AS A MEANS OF DISTINGUISHING LINKAGE GROUPS IN MAIZE[1]

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THE determination of linkage groups in maize is attended with difficulties not met in Drosophila. Of these, the large number of Chromosomes (ten), the relatively few genes whose *loci* have been established and the long developmental cycle—only one or at least two generations can be grown in a year—are not without importance. But the greatest difficulty comes from the fact that crossing over occurs with approximately the same frequency in both microspore and megaspore development. (Emerson and Hutchison, 1921; Eyster, 1922). Where, as in Drosophila, there is no crossing over in the male, linkage, even of genes widely separated on a chromosome, can be determined readily. With maize, however, very loose linkages can not be positively identified as such until one or more other genes with intermediate loci are discovered. Since nothing is as yet known as to the length of maize chromosomes in terms of crossover units, it follows that some of the linkage groups which are now regarded as distinct may in reality pertain to widely separated regions of a single chromosome.

A method which, I am inclined to believe, affords critical evidence for the separation of certain linkage groups in maize has been found through studies of aberrant endosperm development. In an earlier paper (Emerson, 1921) it was shown that, with seeds which are heterozygous for the linked genes *C c* for Aleurone color and *Wx wx* for endosperm texture, spots of colorless aleurone are in the great majority of cases underlaid by waxy endosperm, whereas spots of colored aleurone are underlaid by starchy endosperm. This relation was found not to hold for aleurone and endosperm genes that were not linked. Thus for *A a* and *Wx wx*, which ordinary genetic tests had indicated were independently inherited, the colored and colorless aleurone spots were underlaid by starchy endosperm. It was concluded,

[1] Paper No. 123. Department of Plant Breeding, Cornell University, Ithaca, New York.

therefore, that such mosaic seeds were due to some aberrant chromosome behavior such as non-disjunction or elimination.

Since the publication of my earlier paper, additional evidence bearing on the problem has been obtained. All the available data with which I am familiar are given in Table I.

TABLE I

ABERRANT SEEDS OF MAIZE INVOLVING LINKED GENES

Item No.	Genotypes of parents		Phenotypes of mosaic seeds		Number of Mosaic seeds	Citation of published data, and pedigree number for new data
	Female	Male	Aberrant part	Normal part		
1	c wx	C Wx	c wx	C Wx	1	Collins 1913
2	c wx	C Wx	c wx	C Wx	55	Emerson 1921, table 1
3	c wx	C Wx	c wx	C Wx	3	Emerson 1921, pp. 414
4	c wx	C Wx	c wx	C Wx	8	11453, 11454, 12293
5	c sh	C Sh	c sh	C Sh	1	Emerson 1921, pp. 415, 416
6	c sh wx	C Sh Wx	c sh wx	C Sh Wx	2	11454, 12293
7	i sh	I Sh	i sh	I Sh	7	11450
8	i wx	I Wx	i wx	I Wx	1	Collins 1913
9	i wx	I Wx	i wx	I Wx	43	11451, 11452
Total					121	
10	i wx	I Wx	i wx + i Wx	I Wx	1	11452
11	i wx	I Wx	i Wx	I Wx	2	11452
12	c wx	C Wx	c Wx	C Wx	3	Emerson 1921, table 1
Total					6	
13	y bh	Y Bh	y bh	Y Bh	10	11458, 13553

Of a total of 127 mosaic seeds (items 1–12 of Table I) involving the aleurone factors *C c* or *I i*, together with one or both of the endosperm factors *Wx wx* and *Sh sh*, known by genetic tests to form a linkage group, 121 seeds (item 1–9) showed an exact correspondence in the aberrant part between the recessive aleurone color and the underlying recessive endosperm character of the female parent, the normal part of the seeds showing in all cases the dominant aleurone and endosperm characters of the male parent. In case of one seed (item 10) the spot of recessive aleurone color was underlaid in part by waxy and in part by corneous endosperm. That is, the correspondence between the recessive aleurone color and the underlying recessive endosperm texture was not exact. Of the remaining five mosaic seeds (items 11, 12) the aberrant spot of recessive aleurone color was underlaid by the dominant endosperm texture. Thus, in the great majority of cases the aleurone and endosperm characters of the *C* linkage group are associated in mosaic seeds.

Similar evidence of the relation of linked characters in mosaic seeds is available for only one other group, namely, that involving the linked genes *Y y* for yellow and white endosperm and *Bh bh* for blotched aleurone-color pattern. In all the ten mosaic seeds observed (item 13 of Table I) the yellow part of the endosperm was overlaid by blotched aleurone and the white part of the endosperm was overlaid by colorless aleurone. There is, therefore, sufficient evidence to indicate that mosaic seeds involving the *Y* linkage group are due to some aberrant chromosome behavior such as non-disjunction or elimination, just as are the great majority of those involving the *C* group.

If the results reported are representative of other chromosomes and if the conclusions drawn from them are sound, it would seem justifiable to use evidence from aberrant endosperm development to determine whether other aleurone and endosperm genes are linked or independently inherited, in short, to determine whether their *loci* are in the same or in different (non-homologous) chromosomes.

Mosaic seeds from crosses involving various aleurone and endosperm characters not known to be linked are listed in Table II.

That the *C* linkage group is distinct from the *Su* group is indicated by the data from 41 mosaic seeds (items 1–7 of Table II). In 14 of these seeds (items 3, 4) the aberrant part had the recessive sugary endosperm, but the overlying aleurone showed the dominant aleurone color, *C*, like that of the normal part. The aberrant part of the other 27 seeds exhibited characters due to one or more of the recessive genes *c, sh, wx,* but had the dominant starchy, *Su,* endosperm throughout. The evidence that the factor pair *Su su* belongs to a different chromosome pair from the one carrying *C c, Sh sh* and *Wx wx* is particularly strong when items 6 and 7 are considered. Here the linked recessives, in one case *c* and *wx* and in the other *c, sh* and *wx* were definitely associated in the aberrant spot, thus showing clearly some chromosome aberration, but the other recessive characters of the female parent due to the genes *su* and *y* were not developed.

That the *C* linkage group is distinct from the *Y* group is similarly shown by the 7 mosaic seeds (items 6–11 of Table II) involving the endosperm-color pair *Y y* and one or more of the aleurone and endosperm pairs *C c, Sh sh* and *Wx wx* of the *C* group. That the *C* and *R* linkage groups are distinct is indicated by 16 mosaic seeds (items 12–14). The independence of

TABLE II

ABERRANT SEEDS OF MAIZE INVOLVING NON-LINKED GENES

Item No.	Genotypes of parents		Phenotypes of mosaic seeds		Number of Mosaic seeds	Citation of published data, and pedigree number for new data
	Female	Male	Aberrant part	Normal part		
1	c su	C Su	c Su	C Su	20	Emerson 1921, table 3
2	c su	C Su	c Su	C Su	2	11458
3	c su	C Su	C su	C Su	5	Emerson 1921, table 3
4	c su	C Su	C su	C Su	9	11453, 11454
5	c su y	C Su Y	C su Y	C Su Y	3	11458
6	c wx su y	C Wx Su Y	c wx Su Y	C Wx Su Y	1	12293
7	c sh wx su y	C Sh Wx Su Y	c sh wx Su Y	Sh Wx Su Y	1	12293
8	c y	C Y	c Y	C Y	2	Emerson 1921, p. 418
9	c y	C Y	c Y	C Y	1	13721
10	c wx y	C Wx Y	c wx Y	C Wx Y	1	11454
11	c sh wx y	C Sh Wx Y	c sh wx Y	C Sh Wx Y	1	11454
12	wx su y r	Wx Su Y R	Wx Su Y r	Wx Su Y R	9	11461
13	wx su r	Wx Su R	Wx Su r	Wx Su R	1	11461
14	wx r	Wx R	Wx r	Wx R	6	Emerson 1921, table 3
15	wx a	Wx A	Wx a	Wx A	12	Emerson 1921, table 3
16	wx pr	Wx Pr	Wx pr	Wx Pr	20	Emerson 1921, table 3
17	wx pr	Wx Pr	Wx pr	Wx Pr	2	11452
18	c wx pr	C Wx Pr	C Wx pr	C Wx Pr	1	10171
19	c pr	C Pr	C pr	C Pr	4	10345, 12445, 13721
20	su y	Su Y	su Y	Su Y	8	11458, 11460
21	su y	Su Y	Su y	Su Y	1	11458
22	su y bh	Su Y Bh	su Y Bh	Su Y Bh	4	12287, 13553
23	su y r	Su Y R	su Y R	Su Y R	6	11459, 11460, 11696
24	su y r	Su Y R	Su Y r	Su Y R	28	11460, 11461
25	su r	Su R	su R	Su R	6	Emerson 1921, table 3
26	su r	Su R	su R	Su R	5	11459
27	su r	Su R	Su r	Su R	13	Emerson 1921, table 3
28	su r	Su R	Su r	Su R	9	11459, 12450
29	su y a	Su Y A	Su Y a	Su Y A	4	11464
30	su y a	Su Y A	su Y A	Su Y A	1	13719
31	su a	Su A	su A	Su A	2	11464
32	su pr	Su Pr	su Pr	Su Pr	2	Emerson 1921, table 3
33	su pr	Su Pr	Su pr	Su Pr	1	Emerson 1921, table 3
34	su pr	Su Pr	Su pr	Su Pr	1	12289
35	su r pr	Su R Pr	Su R pr	Su R Pr	1	12450
36	y a	Y A	Y a	Y A	16	11465, 11466, 12290, 13719
37	y pr	Y Pr	Y pr	Y Pr	2	11466
38	a pr	A Pr	A pr	A Pr	9	11690, 11691, 13720, F207
Total					220	

the *C* and *A* groups is shown by the 12 mosaic seeds (item 15) involving *Wx wx* and *A a*. The 27 mosaic seeds involving the aleurone-color pair *Pr pr* and one or both of the pairs *C c* and *Wx wx* (items 16–19) indicate that the aleurone pair *Pr pr* does not belong with the *C* group.

The independence of the *Su* and *Y* groups is shown by 23

mosaic seeds (items 5, 20–23, 30, of Table II) all involving the factor pair *Su su* and one or both of the pairs *Y y* and *Bh bh* of the *Y* group. The 77 mosaic seeds involving *Su su* and *R r* (items 12, 13, 23–28) show independent inheritance between the *Su* and the *R* groups. The evidence that the *Su* and the *A* groups are distinct is limited to 7 mosaic seeds (items 29–31). The 5 mosaic seeds (items 32–35) available to show the relation of the pairs *Pr pr* to the *Su* group indicate independence.

That the *Y* and *R* groups are distinct is shown by 37 mosaic seeds (items 12, 24, of Table II). The independence of the *Y* and the *A* groups is indicated by 20 seeds (items 29, 36). Only two mosaic seeds (item 37) have been found to involve *Y y* and *Pr pr*. These two give no indications that the pair *Pr pr* belongs to the *Y* group.

That *Pr pr* does not belong to the *A* group is indicated by 9 mosaic seeds (item 38 of Table II). Only one mosaic seed (item 35) is available as a test of the relation of *Pr pr* to the *R* group and this gives no indication of linkage. If *Pr* were linked with *R r* or with *A a*, no direct evidence of the fact could be obtained from mosaic seeds. When either recessive *r* or recessive *a* is homozygous, neither purple nor red aleurone color develops, and it is therefore impossible to determine whether *Pr* or *pr* is present in an aberrant spot.

For the same reason it is impossible to obtain evidence of the relation of *R r* to *A a* by the mosaic-seed method. Obviously, when *r* is homozygous, the lack of aleurone color thus assured makes it impossible to determine whether *A* or *a* is present. And, similarly, homozygous *a* makes it impossible to determine the presence of *R* or *r*. It will be possible to test the relation of *R r* to *A a* only when, if ever, there is discovered some other endosperm factor linked with one or the other of these factor pairs, as *Sh sh* and *Wx wx* are with the aleurone pairs *C c* and *I i*.

By way of summary, it is to be noted that there have been made all the comparisons that are possible at present, by means of the aberrant-endosperm method, to determine the linkage relations of the several aleurone and endosperm factor pairs of maize. True, it can not be claimed that wholly adequate data have been obtained for all these comparisons. It may fairly be concluded, however, that the 137 mosaic seeds recorded in Table I afford strong evidence that, with respect to the characters belonging to the *C* and to the *Y* linkage groups, mosaic seeds are due to some

aberrant chromosome behavior such as non-disjunction or elimination. If mosaic seeds involving other endosperm characters are due to the same behavior of other chromosomes, the data reported in Table II for 220 mosaic seeds may be used in an attempt to determine whether certain linkage groups are actually distinct. The extent of these data is shown in Table III.

TABLE III

SUMMARY OF THE NUMBERS OF MOSAIC SEEDS OBSERVED IN TESTS INDICATING INDEPENDENT INHERITANCE OF CERTAIN LINKAGE GROUPS IN MAIZE

Linkage Groups	*Su*	*Y*	*R*	*A*	*Pr*
C	41	7	16	12	27
Su		23	77	7	5
Y			37	20	2
R				0*	1
A					9

Except for the relation of the *R* group to the *A* group, which can not be tested by the mosaic-seed method, the evidence in most cases is sufficiently extensive to indicate that the *C*, *Su*, *Y*, *R* and *A* linkage groups are distinct. The evidence is fairly good also that the *Pr pr* factor pair does not belong to either the *C*, *Su* or *A* groups, and there is some evidence that it does not belong to either the *Y* or the *R* group. It is noteworthy that in no instance was there observed a single mosaic seed that suggested linkage between any of the groups here under consideration.

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* No tests are possible (see text).

SHORTER ARTICLES AND DISCUSSION

THE VARIABILITY IN WEIGHT OF LEGHORN CHICKENS AT HATCHING, THIRTY-FIVE DAYS AND MATURITY[1]

THERE have been many studies upon the growth of the chicken and yet none, so far as I am aware, have given any information concerning the variability of the chick at different ages, nor the correlation between the weights at different periods of growth. This little study which is based upon nearly 2,000 weighings is an attempt to give some information upon these two questions.

The chickens used were single-comb White Leghorns which were hatched and raised by the department of poultry husbandry of the University of Nebraska. The weighings were made in connection with another problem, but these weights were turned over to me by Professor Frank E. Mussehl and I wish to thank him most heartily for his kindness in placing these data at my disposal. I wish to thank Miss Lois Pedersen who helped in making some of the computations.

These chickens were artificially hatched and reared. They were hatched at various times between February 12 and May 11, 1922. Each chick was marked by a wing band and later by a leg band, so the weight of each individual chick is available for each period.

There were 510 males and 452 females weighed at time of hatching, 414 males and 379 females of the above chicks were weighed when they were 35 days old and 239 of the females were weighed when they began to lay, thus making a total of 1,994 weighings. The age at the time the first egg was laid was determined for 263 of these pullets. The pullets were trapnested and the weight of 239 of the 263 was obtained before they were released. The weighings at the time of hatching and at 35 days were accurate to one gram and the weighings at the beginning of laying were made in pounds and accurate to a quarter of a pound. These weights have been changed to grams to correspond to the other weights.

[1] Studies from the Zoological and Anatomical Laboratories of the University of Nebraska, No. 138.

The average for the weights of the males and females at hatching and at 35 days and for the females at time of sexual maturity and also the average age of sexual maturity is shown in the first line of Table I. The second line shows the range, the third the standard deviation and its probable error and the fourth line shows the coefficient of variability and its probable error for each weight and the age (last column) at the time the first egg was laid. The first two columns show the hatching weight, in grams, of the 510 males and the 452 females, the next two columns show the weight at 35 days of the 414 males and the 379 females, the fifth column shows the weight in grams of the 239 females at the time they began laying. The last column shows the age at which the 263 females began laying. The formulae used in determining the standard deviation, the coefficient of variability and the coefficient of correlation and the probable error of each are those given by Davenport.[2]

The data upon the growth of the Leghorn chicken given by Buckner and associates,[3] and Card and Kirkpatrick[4] have been plotted in connection with similar data collected by Latimer,[5] and the averages for the weights of these chicks at the three periods when plotted on the same chart are found to be very close to the other curves for the first two periods and are slightly heavier than the chickens reported by Latimer at 210 days. Buckner and associates carried their study only to the end of the 24th week and Card and Kirkpatrick carried their experiment to the end of the 28th week.

The coefficients of variability which are the best measures of the amount of variation are shown in the last line of Table I. These show that the chickens are the most uniform in weight at time of hatching, and the least uniform at 35 days. The coefficient of variability for the females at hatching being 8.09 ± 0.18 and at 35 days of age, 21.78 ± 0.56, or 2.69 times the coefficient at time of hatching. The coefficient of variability for the weight at the beginning of laying is 11.81 ± 5.97 or 1.46 times the coefficient of the hatching weight of the females. Thus, these chickens show the maximum variability in weight at 35 days, less variability at sexual maturity and the minimum, at time of hatching.

[2] "Statistical Methods," John Wiley and Sons (1904).

[3] *Am. Jour. Physiol.*, Vol. 47, pp. 393–398 (1918).

[4] Bull. Storrs Exp. Sta. No. 96 (1918).

[5] Unpublished Material. Abstract, Vol 2, Papers from the Mayo Foundation and the Medical School, University of Minnesota.

The chick at 35 days has passed its initial period of slow growth, including the first three or four days during which, like the human, its weight is less than at the time of hatching, and by this time it has begun to grow rapidly. Just before sexual maturity, as shown by the beginning of laying in the pullets, the growth curve flattens out, indicating a retardation of the growth rate. Thus, at hatching and at sexual maturity the growth rate is less rapid and the variability in weight is less than at 35 days of age when the rate of growth is very rapid. This shows that in chickens as well as other animals, in which this has been studied, a period of rapid growth is a period of greater variability. Unfortunately, the available data give no means of determining just when this maximum variability occurs in the growth of the chicken.

Jackson[6] in his study of the variability in the weight of the albino rat reports the lowest coefficient of variability (12.3 ± 0.64) at birth. This rises to a maximum of 28.4 ± 2.1 at 20 days of age and then drops to 19.1 ± 1.5 for 5 months of age. These coefficients of variability are for both sexes combined. Porter[7] in his study of St. Louis school children finds the maximum variability in weight of girls at 13 and 14 years and at 16, for the boys.

The coefficients of variability for both the males and females at hatching and at 35 days are shown separately in Table I. The coefficient of variability is shown for the females only at time of the first egg. The females are slightly more variable at hatching, the coefficient of variability being 1.097 times as large as that of the males. At 35 days of age this condition is reversed and the males have a coefficient of variability 1.05 times greater than that of the females.

Jackson finds the coefficient of variability for the male albino rats, at all ages, greater than that for the females except at 6 weeks, when the females have a coefficient of variability 1.4 times as great as that for the males. Hatai[8] finds the coefficient of variability of the adult male albino rat about 2.1 times that for the female, or 25.076 ± 2.675 and 12.235 ± 0.974, respectively. Porter finds a greater variability in the weight of the girls from 6 to 13 years inclusive, and from 14 to 17 years inclusive the boys show the greater variability.

[6] *Am. Journal Anat.*, Vol. 15, pp. 1–68 (1913).

[7] Trans. St. Louis Acad. Sci., Vol. 6, pp. 233–250 and 263–426 (1894).

[8] *Am. Jour. Anat.*, Vol. 7, pp. 423–441 (1908).

TABLE I

	Hatching weight		35-day weight		Mature weight females (239)	Age (days) mature females (263)
	males (510)	females (452)	males (414)	females (379)		
Average	39.45 ± 0.09	39.16 ± 0.10	209.15 ± 1.60	191.71 ± 1.45	1626.51 ± 8.38	209.81 ± 1.09
Range	32–50	30–48	66–351	83–297	1140–2268	153–303
Standard deviation	2.91 ± 0.06	3.17 ± 0.07	48.39 ± 1.13	41.75 ± 1.02	192.14 ± 5.93	26.27 ± 0.77
Coef. of variability	7.37 ± 0.16	8.09 ± 0.18	23.14 ± 0.54	21.78 ± 0.56	11.81 ± 5.97	12.52 ± 0.78

TABLE II

Coefficients of correlation	
Hatching weight and 35-day weight—Males	+ 0.2202 ± 0.0259
Hatching weight and 35-day weight—Females	+ 0.1042 ± 0.0344
Weight at 35 days and maturity—Females	+ 0.0061 ± 0.0436
Weight at 35 days and age at maturity—Females	— 0.2672 ± 0.0407

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Correlations: The data at hand give the weight of the individual chickens at two periods for the males, namely, at hatching and at 35 days, and for three periods for the females, at hatching, 35 days and at the beginning of laying and so these data have been used to find out to what extent larger chicks at hatching are large chicks at 35 days and also what relationship there is between the weight of a 35-day-old female chick and the same chicken at the beginning of the laying period. A statement frequently made by poultrymen is that the best laying pullets begin to lay at an early age and so an attempt has been made to determine the relationship between weight at 35 days and the age at which the pullet began to lay.

Table II shows the coefficients of correlation between hatching weight and weight at 35 days for both the males and females. The third correlation is between the weights at 35 days and at the time of sexual maturity, and the last correlation is between the 35-day weight of the females and the age at the time the first egg was laid.

The first two correlations ($+ 0.22$ and $+ 0.10$) are so low that they are not significant. In other words, there is very little probability that a large chick, at hatching, either male or female, will grow into a large chick at 35 days. Similarly, there is practically no correlation between the 35-day weight and the weight at time of sexual maturity. This correlation is but $+ 0.006$ and its probable error is ± 0.044, or over seven times the correlation, so this may be considered as of no significance. The correlation between weight at 35 days and age at which the pullet began laying shows a slightly higher correlation, although it is a negative correlation. This correlation is the highest numerically of any of the correlations, and so this would indicate that there is a greater probability of a large female chick at 35 days beginning to lay earlier than the average. This is, however, not high enough to be called a significant correlation. The commercial poultryman would be delighted if he could pick the most productive pullets at an early age and market the others together with the surplus cockerels at the end of the maximum growth period, but the above correlations do not indicate a sure method for making such a selection. A correlation between the body weight a little later on and the age of sexual maturity might give a more significant correlation, but the data at hand does not justify any predictions of the size or age of sexual maturity based on the weight at 35 days.

SELECTIVE ACTION OF STRYCHNIN AND NICOTIN ON A SINGLE CELL[1]

THE greater amount of work done on the action of drugs on unicellular forms concerns the problem of efficiency of antiseptic and disinfectant drugs of various concentrations. There is a relatively small number of investigations of other types. The observations to be reported in the present paper were obtained by applying strychnin and nicotin in different concentrations to some of the smaller ciliates which developed in a hay infusion prepared in the laboratory. The form to which special attention was given is *chilodon megalotrocha,* Stokes. (See Figures 1 and 2).

The results show that strychnin and nicotin act selectively on certain portions of the one-celled animal. This can be shown by applying the drug in varying concentrations. It is difficult to understand this selective action on the single cell unless we consider that, as Rees[2] has demonstrated, the infusorian possesses structures which are comparable to ordinary neuro-muscular mechanisms. Rees has shown clearly that the paramecium possesses a neuro-motor apparatus. The nervous elements at the periphery consist of fine branching fibrils. These converge to a neuro-motor center. At the periphery these are connected with the basal granules of the cilia. If we accept the statements made by some investigators that the cilia are the ends of myoid fibrils and that the physiological behavior of these elements is similar to that of the fibrils of skeletal muscle, we may say that the selective action of drugs involves the idea of mutual antagonism or inhibition among the fibrils of the different portions of the body of the ciliate.

I find it profitable to consider that the neuro-motor center inhibits or correlates the action of a number of more or less independent functional units of the cell, and that this correlation is responsible for the typical movements of the animal in swimming—this is a forward movement with a greater tendency to turn to the left than to the right. There is also a tendency of the animal to turn over and over on its long axis as it swims, but this occurs very seldom under normal conditions. The functional units of the animal differ chemically among themselves,

[1] From the Department of Physiology and Pharmacology, Marquette University Medical School, Milwaukee, Wisconsin.

[2] Rees, C. W., AMERICAN NATURALIST, 1921, *55,* 464–469.

the criterion being the selective action of certain drugs. Certain drugs affect some parts of the cell more than others, and this results in the special forms of reaction of the animal which are to be reported.

When a drop of the water containing the animals is treated with either a solution of strychnin or nicotin, the animals generally assemble and remain in small groups. Presumably, this is due to a greater chemical affinity existing between them after the drug is applied. A special factor responsible for the perceptible reactions attributed here to the increase in chemical affinity is the motility of the animals. If the individuals tend to collect anyway, because of the addition of the drug, any movement of the cilia will only hasten the process.

After this grouping occurs, the animals begin to respond in one specific way—those treated with a drop of 1: 1000 solution of strychnin sulphate rotate in the counter-clockwise direction with the posterior in advance of the anterior end. It is best to apply the solution along one side of the coverglass and allow it to become distributed gradually throughout the water. The oral groove is to the right of the animal as long as the turning in this direction lasts. Figure 1 represents the position of the animal during this time. The curved arrow, S, indicates the direction of movement due to strychnin. A indicates the anterior and P the posterior end of the animal. Figure 1 represents the animal in its normal position; it is here resting on its ventral surface. However, a specimen is occasionally found resting on its dorsal surface, as is shown in Figure 2.

From the time that the 1: 1000 solution of strychnin sulphate is applied until the animals die, the amount of activity becomes gradually less. When the turning first begins the anterior end of the body is almost stationary, if not entirely so, but after a few minutes of turning each animal describes large circles by moving very slowly, posterior end first, in the counter-clockwise direction. The change from the small to the relatively large circles is a gradual process. We may profitably assume that the drug affects certain portions of the cell more than others. The circular or spiral movement would be due, then, to the selective action of the drug upon the organism. The strychnin depresses all the cilia, affecting those on the right more than those on the left. It is also true that the anterior cilia are depressed, but less than those on the right or left. The posterior cilia are depressed

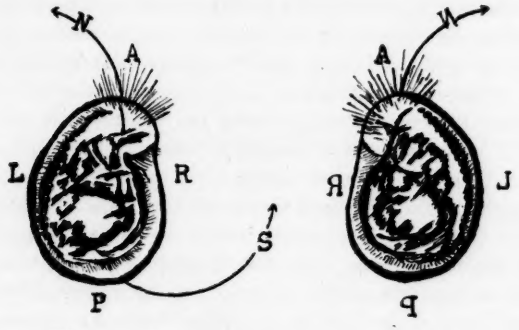

FIG. 1 FIG. 2

FIG. 1. Animal resting on ventral surface. L, left side; R, right side; A, anterior end; P, posterior end; S, direction of movement due to strychnin; N, direction of movement due to nicotin.

FIG. 2. Animal resting on dorsal surface, which is one stage of nicotin poisoning. The letters (inverted) have the same meanings as in Fig. 1.

least of all. It could not be observed that strychnin increased the action of any of the cilia; depressing effects only were observed. The rotation in the counter-clockwise direction with the posterior end first is due to the relatively great activity of the posterior cilia which execute their rapid or driving strokes from left to right. The strychnin either decreases the action or leaves functionless the cilia of the right side, left side and anterior end; and since those at the posterior end are still relatively very active these necessarily turn the animal in the direction indicated by the curved arrow S. As the cilia on the right side, left side and anterior end become less and less active the animal ceases gradually to make the small sharp turns resulting in very small circles and makes the larger ones which cease only when the cilia at the posterior end also become inactive.

When a 1: 1000 solution of nicotin is applied along the sides of the coverglass and allowed to become distributed gradually throughout the water, the animals first form groups and then each of them turns in the counter-clockwise direction similarly as when strychnin is applied. In this instance, however, the animal moves forward instead of backward. The direction of movement is indicated by the curved arrow N in Figure 1. This difference is due to the fact that in nicotin poisoning the posterior cilia are depressed sooner than the anterior cilia. These anterior cilia make their rapid or driving strokes from left to right, which

results not only in pulling the cell forward but also in continually forcing the anterior end to the left. After a time, however, a certain irregularity in the movements of each of the animals occurs, but this does not mean an increase in the activity. The animal now swims forward, turning on its long axis as it goes. Finally it comes to a standstill, but it is now resting on its dorsal surface. The oral groove is on the left instead of on the right side of the animal. Figure 2 shows this abnormal position. The cilia seem to be functioning too weakly to cause any movement of the body as a whole. In a few seconds, however, the animal begins to turn in the clockwise direction, the posterior end being almost if not entirely stationary. Small circular movements of the entire body develop, and these become gradually larger, until the anterior cilia cease beating and the animal dies.

The turning on the long axis as the animal swims is due to the persistent action of the cilia near the mouth of the oral groove, after other cilia in the groove and elsewhere on the body have stopped beating. It can be observed easily that these large cilia are the last of those in the groove to stop beating, and that when the animal is resting on its ventral surface these cilia execute their rapid or driving strokes in the downward and slightly backward direction. This causes the animal to rotate on its long axis and to move at the same time in the counter-clockwise direction. As soon as these cilia weaken or cease beating entirely the animal comes to rest, generally on its dorsal surface. It is after this that the anterior cilia, which are still active, drive the animal in a spiral fashion in the clockwise direction when viewed from the ventral surface or in the counter-clockwise direction when viewed from the dorsal surface. When the animals are observed from their ventral surfaces, the spiral movement is in the clockwise direction with respect to the position of the observer. However, if we imagine ourselves observing the inverted animals from their dorsal surfaces, *i.e.*, from underneath the microscope, they would be turning in the counter-clockwise direction as usual.

The turning on the long axis does not occur in strychnin poisoning because the cilia of the groove are among the first to be paralyzed. This is, therefore, only another special observation concerning the selective action of drugs on the single-celled animal.

SUMMARY

(1) The animals show a tendency to collect in small groups soon after strychnin or nicotin is applied.

(2) Strychnin acts specifically on the body of the animal, selecting and depressing especially the locomotor organs on the right and at the anterior end of the cell. The posterior cilia and the still active cilia on the left then turn the animal in the counter-clockwise direction, with the posterior in advance of the anterior end. Eventually, all the cilia are paralyzed. The animals remain on their ventral surfaces.

(3) Nicotin also acts selectively on the body of the animal, depressing especially the posterior cilia and those on the left side of the cell. The only cilia on the right which are not depressed early are the long ones at the mouth of the oral groove. As long as an animal remains on its ventral surface, these cilia of the groove and the still active ones at the extreme anterior end turn the cell in the counter-clockwise direction with the anterior in advance of the posterior end.

(4) Some time after nicotin is applied the animals cease turning in the counter-clockwise direction, respond irregularly for a short time, and eventually go forward turning over and over as they go. This turning of an animal on its long axis is due to the action of the cilia at the mouth of the groove which normally tend to cause the organism to rotate in this way, but which ordinarily fail to cause the rotation because other cilia (which are now depressed) overcome the effects of the groove cilia and keep the animal on its ventral surface. It is only after certain cilia, presumably those on the left side, are paralyzed that the cilia of the groove can turn the animal as they normally tend to do. The turning over and over as the poisoned animals go forward ceases finally, as the cilia of the groove become depressed, and then the great majority of the animals come to rest on their dorsal surfaces. After this the animals turn again in the spiral fashion in the counter-clockwise direction with respect to their dorsal surfaces. All the cilia are eventually paralyzed.

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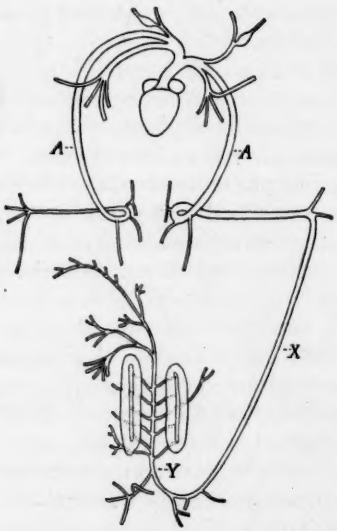
ABNORMAL CIRCULATORY SYSTEM OF A FROG

RECENTLY a frog (*Rana pipiens*) was found with such an abnormal circulatory system that it seems worthy of record. In so far as I was able to discover the venous system was normal in all respects.

The accompanying diagram shows the arrangement of the main arterial trunks. The chief difference between this individual and the normal frog is that the systemic arches (A, A) never unite to form a dorsal aorta. The blood for the abdominal viscera and the posterior part of the body is supplied instead by a large trunk (X) from the subclavian. This trunk joins at the posterior end with a vessel (Y) which is the same as the dorsal aorta in its position and which supplies the same parts of the body as the latter. It differs from it, however, in that it is not formed by the fusion of the systemic arches and that in it the blood flows in an anterior instead of a posterior direction as is usual in the aorta. The liver has no arterial blood supply. The systemic arches



(A, A) are present and near their origin appear normal but neither extends posterior to the subclavian except as small, rudimentary vessels which never join but lie beside the vertebral column. The vessel X which replaces the aorta is not in the abdominal cavity but passes outside the peritoneum and lies dorsal to the spinal nerves.

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